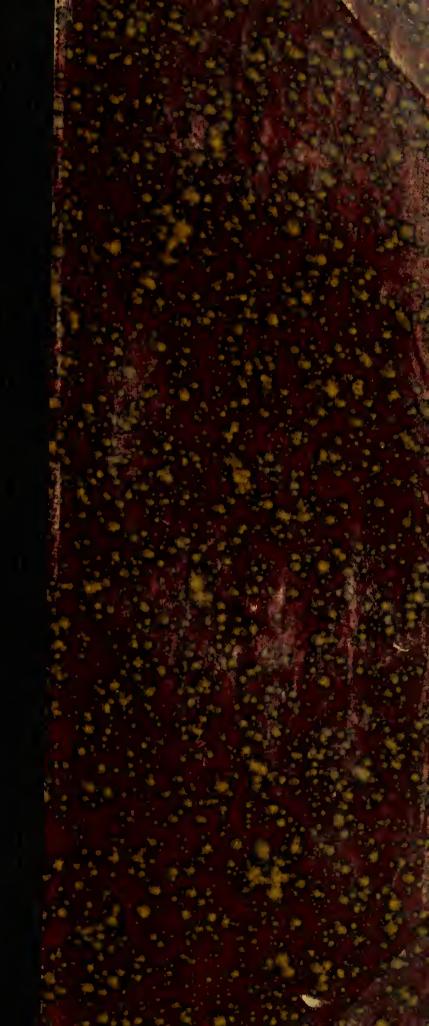
# **ZUCKER**

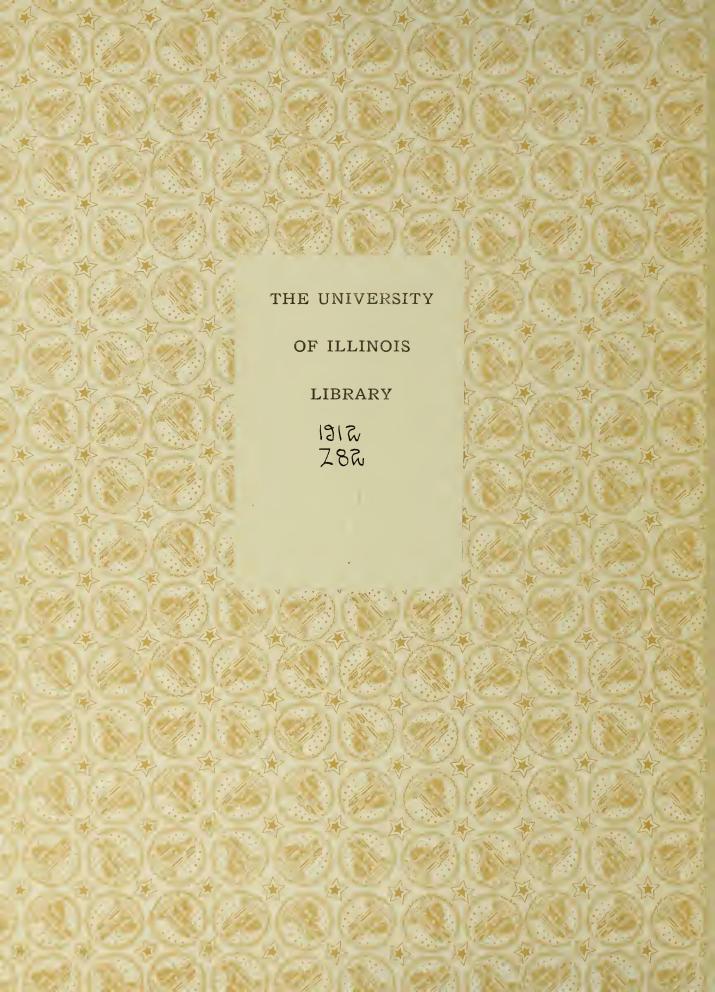
Studies on Amylases

Physiological Chemistry

M. S.

1912









### STUDIES ON AMYLASES

BY

THEODORE FREDERICK ZUCKER

Concordia College, 1907

### THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

IN PHYSIOLOGICAL CHEMISTRY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1912

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## UNIVERSITY OF ILLINOIS THE GRADUATE SCHOOL

May 31, 19012.

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Theodore Fredrick Zucker

ENTITLED

Studies on Amylases

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF

Master of Science

In Charge of Major Work

Outfar

Head of Department

Recommendation concurred in:

Committee

on

Final Examination



STUDIES IN ACILASES.

The Specific of Amylase and Maltase.

Part I. Significate of the problem and discretion of the little tree.

Part II. Experimental work.

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### STUDIES ON AMYLASES

The Smidification' Amylere and Maltase.

Part. I. Simifacence of the problem and

Part II. Experimental work.

#### PART I

It is is ally said that of all enzymes the diastases,

i. c., the anylol, the and olycomenolytic enzymes are the best

beginning and at extensively studied. In soite of all the work

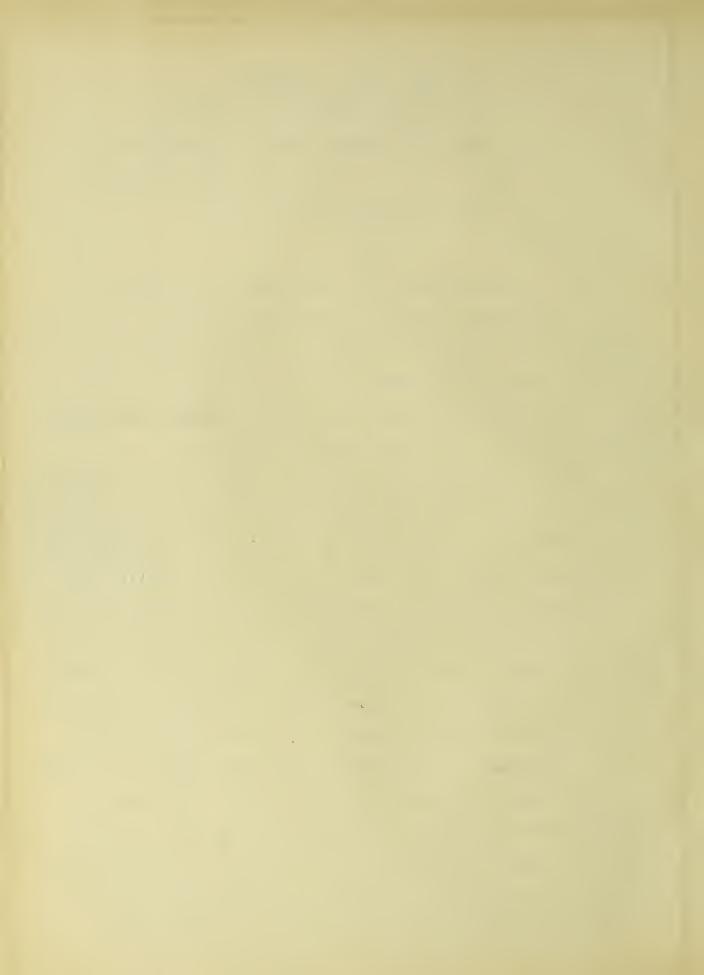
lone on these enzymes there are still a number of ouestions of

most fundamental apportance valor have not at all been decided

estimatorily or have been hardly touched upon.

Most curprising of all is the fact that there is not one withod which is of comewhat general applicability and sufficient accuracy for the quantitative estimation of the action of the amylolytic engymes. The reason for this lies chiefly in the fact that starch and algorithm and their decomposition products are not well amount characterized to allow of any easy separation and quantitative determination. Besides this, the difficulties met with in the preparation of representative samples of amylolytic engymes are enumber on account of the complex that note which even small quantities of inormanic material mays in the "activation" or acceleration of enzymic action.

Since the importance of these such most has only lately been fully recommised, much of the older work has become doubtful, and the present literature is full of contradictions.



2

Another very import not topic remarding which we are still mery much in the dark is the accurate characterisation of similar enzymes. The name of names of enzymes has increased enormally in lite years and is still increasing. This fact alone is liable to raise the mustion in one's mind: Do animal and plant tissues mally synthesize so many specific hories of an enzyme nature which are specifically adjusted to a substrate which, in some cases does not at any time occur within those tissues? (70)

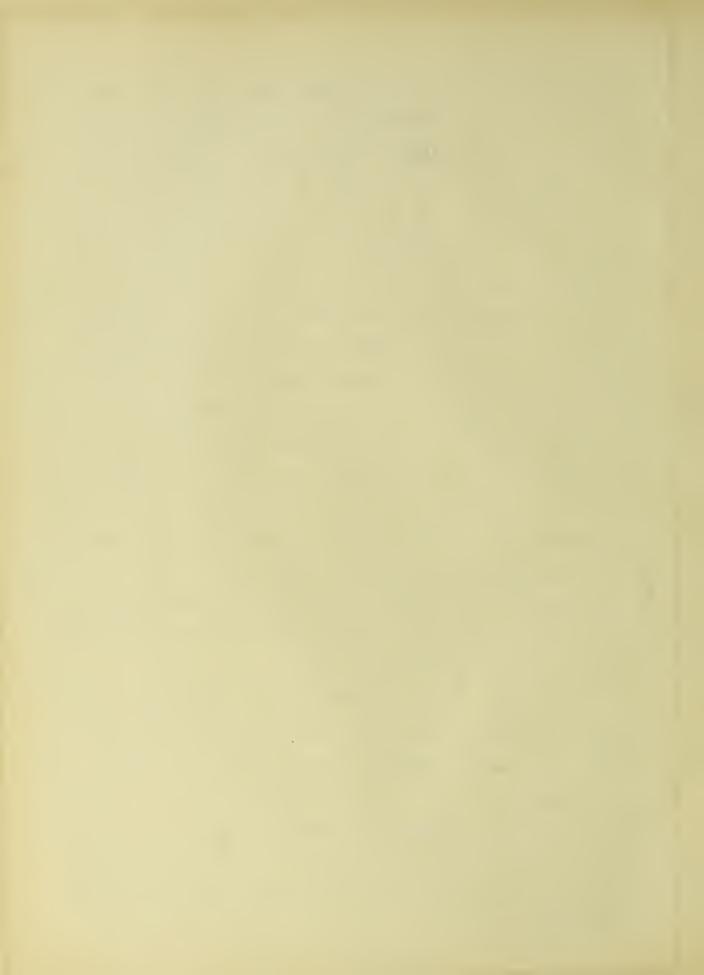
A case where the question of specificity is of great interact is in the diastages of the animal body. The question here is principally: Does the liver build u, for the purpose of learner or its olycomen, a co-cific plycogen enzyme ( a Tycorenase) ? The income of this question is seen for instance in the transfer of Macleod (1) on Tycosuria. In the sum-- Fr of 19 9 Prof. Pacleod suggested this problem to Dr. Haskins on the writer. This work was later interrupted and the writer took up in 'enendertly anot' er question which is more furdamental and presents less difficulties than the original one: In the engyma ic hydrolysis of sterch or alycogen to restrose is it necessary to assume two spucific enzymes, one for the transfor stion of to pulysaccharice to disaccharide end other for " a hydrolysis of dispectoride to monosaccharine? (" e greation of sever polycaccharide enzymes is isregarded for the present).

If it can be established that maltase action and amplase action are not due to two specific enzymes then, of course, there is no reason for looking for specific enzymes for starch



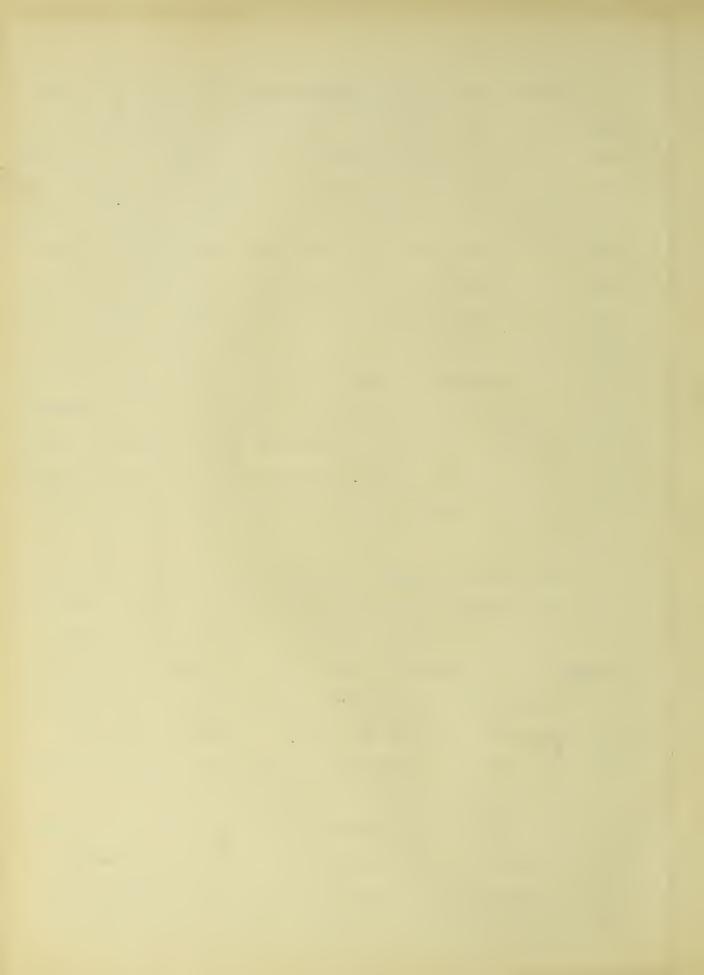
and for allower. If, on the other land, there are really two diffice t enzymer resonsible for the hydrolysis of polysaco mile o describacide and disaccharine to monosacolatice, then this should be the improvement in the qualitative determinotion of liant se. It is evin t that a removal of the maltone tip the ydrolysis of a polysaccharide by the action n another engyme must affect the velocity of the first reaction. Lee (2) has remonstrated the retarding effect of products of the raction is the case of amylase action and Philoche (3) and elso Arnatrone (4) ir the case of maltase action. Therefore in disections of starch or clycomen, in which dextrose is produced the rate of conversion of Je molysaccharide will be at ected by that of the importantice, and if there are the enzymes which to many intenendently, t on the rate of disappear ace of the rolysech erile is not an accurate measure of the "concentration of the diactase", but will be influenced by the amount of maltase Which happens to he present. The interpretation of a case in which amylase action and maltase action do vary independently will he iven in a later para rap'i.

Besides all this an investigation of specificity is of memberal hipphemical interact. It is customary to look upon the fiscovery of a new enzymatic process as croof for another distinct enzyme. I holds find that two different enzymatic processes do not postulate two specific enzymes, it would modify our conception of these processes considerably and would throw new limit on the role which enzymes play in the life processes.



tase her hear fully setting. Pischer's lock as lev simile had become so north that the conjection of an engine as a catallat specifically adjusts to a particular substrate was applied to analysis. It was very natural to the early workers in this field when they observed that some engine preparations changed starch into maltose and others channed it into dextrose, to conclude that this was due to two different engines. There was no reason not to assure this. It is only after we have learned that other substances, to ides the engine itself, play an extremely important part in enginetic processes that the prestion assessments the ability of mind or plant substance to catallyze a given channel channel, does not depend just as much upon other materials present as it does upon structure of the engine molecule.

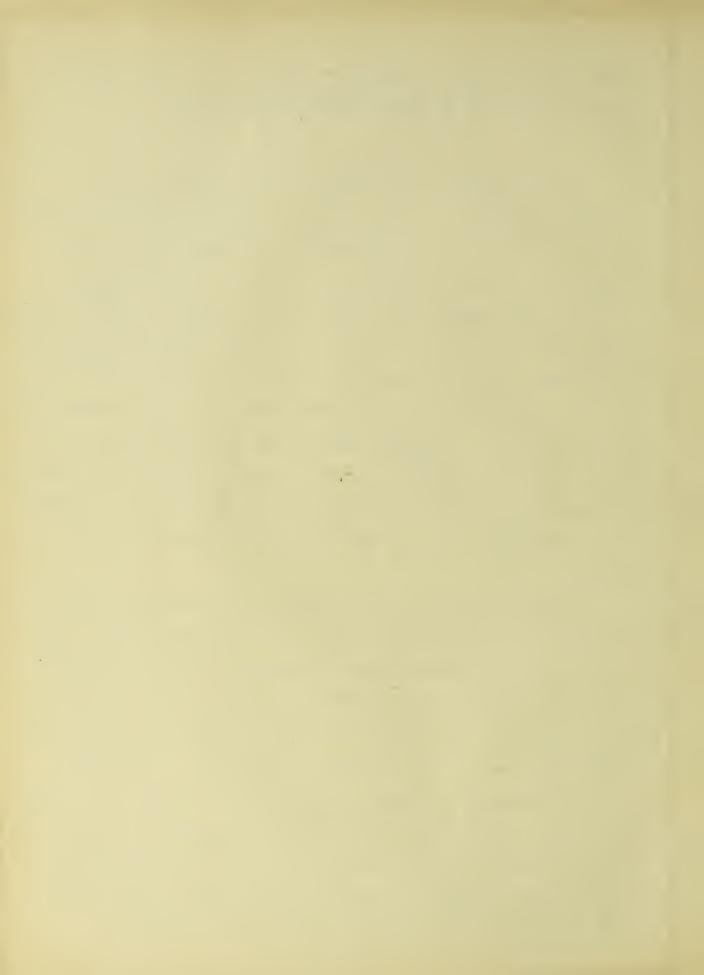
To be not the early literature on maltase, we find that with those authors the diestion usually was not, whether they were decline with a specific enzyme but whether dextrose was produce by the enzyme they had. This is why Samuely (5) can say of maltase: "Der Tachweis des Fermentes ermis t sich aus dem Chemismus seiner Wirkum var celbst." Vernon, (6) and also Enler, (7) in the maltase in their monocraphs on enzymes, so not must any evidence for the maltase action being due to a specific marme. Openheimer (8), after cities the evidence from it forward by various authors for a specific maltase, says that in 1895 Fischer "Accided the quantion definitely by means of the opazone reaction". Therefore Openheimer is a sees the



nrong a specific maltage in the fact that and employed marketions produce lextrone no others do not.

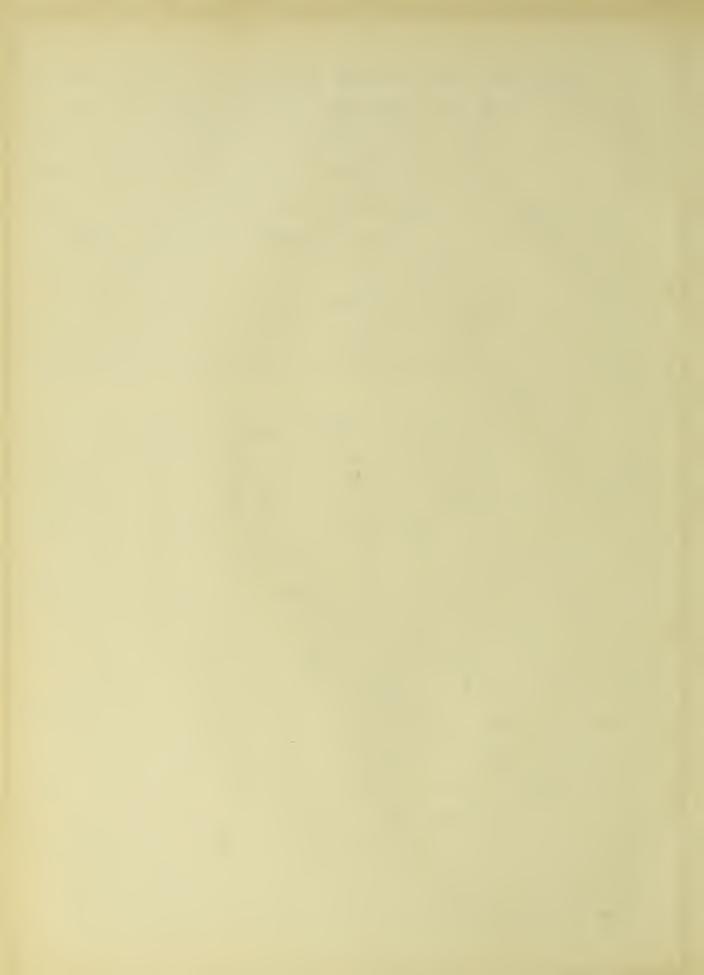
specific moltage, the mitter means to call attention to the fact most be made to find in the literature a single instance in which halt he action is exerted without amylase action, i.e., wherever "maltase" occurs, "amylase" is also present. Taylor (9) claims that certain on the lower moltage evidence of maltage without amylase but to lose not give any references. In all those cosmo, (No he discussed later) in which "maltage is were considerable extent. We see, therefore, that in no case has the separation of two engines been accomplished. An engine, which hydrolyges stanch, always hydrolyges maltose and vice were.

Textrools usually say that salive and cancer tic juice bydrolyse starch to maltose and no dextrose is produced. But under material conditions these secretions do poduce dextrose. Hamburger (/0) has dominated the maltase of coduce dextrose. Hamburger (/0) has dominated the maltase of coduce dextrose. The folial, while main quantities of saliva ind not produce fextrose, there was no difficulty in obtaining it by using leaver quantities. Pierry (//w) and co-workers found that normal concretic juice of their deby secreting was alkaline and strongly amplolytic but did not hydrolyze maltose. As so we as it was neutrolized it exhibited marked maltase office. Here again we see that amplace and maltase office hand in hand.



Semuely (5) quotes the worst of More lus and Merin (3) as
the first evidence for "Miltage". These authors studied the
lydrolysis of starch and of ricco en by diastase of malt, saliva,
monoress and over. They directed larne quantities of the polysaccharide and the remarkand and purished the products. They
found that after a long cripl of direction dextrose, besides
achroolextrin and maltose, was formed by all the enzymes. This
article is direct of mainst Nasse who hald that these enzymes
to not produce fextrose. Musculus and Millage do not say a word
about a medicic enzyme but merely live evidence of the production of dextrose.

The instruction of a specific raltage is made by Chisirier. In 1305 Chicinier had a process patented for Line hr rich in a great, said to be "cerealose". Then In 1891 he and e was are, Gebuld, (14) found that the sugar was profiteed by a special engyme which they called "clucase". They had in the matime recognized it as alucuse. What their process was, foes not seem to be given in the cheminal literature but in 18 6 Cuirinier (15) published a paper on "glucase" and the "seccharification" of starch. His arounents for a specific cluance are about the following. The often described properties of molt directase do not surfice for the explanation of the Totefaction of starch in plants, especially in the grain, for is the columbe part of this he examined it is sound that the cuser contain d in it is principally dextrose. How does this if the arear proction is due to diastage in the seed agree with the rell-known property s of disstage to convert starch



7

into 'ext ne ar \_\_tuso? We not either our e that diastase mis 'illere ti on intot starch cranles tar it was on ture' of the or that him and on this a special instase, which has this liming power. Te to ites experimental or the latter view. Has experiments show the latter view. finely ground and i set a tamperature of 40-50 ce-- (le'ow t'e temperature at which a paste is formed), the ctarch is "ious"is and or as to Clique action is complete the irest contains dextrose. In this experi et the liquefaction "se viry slow "it more complete ( i. e., /extrose was formed) t in whom starch nacte was used. If unrerminated grain was used the process use still slower and dextrose was again formed. In another set experiments he noticed that in starc's paste Transfer equantity of malt the light action was remined and liqueried, the digest contained maltose (bemides dextrins) but no dextrose. If, on the other hand, a small cuantity of malt was used, the liquefaction was slow and when lin efied, he disset on tained largely dextrose.

Such a liter are not becoming to the enzymatic process,

Let able to ined with acid as hydrolyzing a lent. Much

less do they offer any swidence for a specific maltase. The

conation is evident that in the short that a mined to

licens: the description of a sculve preparation is used, the maltase and in the continuous section ent, but when the liquid action

requires a location time dext one in formed.

Word (%) 'se observed . It in the hydrolygis o' sucreh by scient ellows accommented in ring the first of the process



and lettr the notation of dextro e increased. That malt will produce is well know. Even before Chisinier, this had been a trated by Musculus and V Morin (/3)

Consider is and off ority liven by Fincher (7) and by Foreign (8) for the existence of a specific maltase. We set that Chimier has offer independence to prove the point but Fincher are Tohmann and after them all others have accepted it. This is not so substitute fine at the time Fincher and Fohmann published the papers the main reasons for questioning the enecificity of maltase were not get known. The mincipal point these courses were not get known. The mincipal point these courses were not get known.

of the advances which have been made in the study of enzymes since 1895 when, as Opnenheimer ways, Fischer "decided the question defintery by more of the osazone reaction".

Instruct a not a, hases, ilts, orbtein and amino acids have then shown to rodify the action of enzyman to materially, either action or even entirely institution them, it is evident at if we indicate preparation not to not an arriven substrate we are not permitted to conclude that it vould not act if the conditions were modified. From we know definitely what all the conditions are we cannot be certain that our apparently logical conclusions are actually true..



A one the primary of the effect of lictrolytes that of Sc 1 200 (19) claims special tention in this con. ention. Solim or atmin to elict of carbon toxi e on the instatic nentone for in enzymes. The results showed that starches of different with had a different directibility with saliva. This e ound resended on the reaction of the starch which was probably due to lifterent a in the proce of manufacture. Wheat steren wich was slightly acid was most easily digester. Next care soco, potato, and careo stero dich were neutral in oction. Rice starch which was alvelie was least divestible. The earne preparation are vas diluted salara. These directions were or ried on in an atmosphere free from carbon dioxide. Parallel experiments in which the air in the flasks had been figh sond by narhon dioxi - showed a very considerable increase in the disection of the climberice torch, a moderate increase in the later laterches, a slight decrease in the acid

. . at starc'. To show what a tremendous effect reaction has

in the amylolytic power, Schierbeck carried out other experi-

marts, one of which wave the following results:

Crutaner (20) called attention to a number of significant facts. He found that in every case the smaller the amount of



This is enclosured the source which Armanius (2/) obtained in the environment of came shear by acids, which show that a given that in the hydrolysis hydro

With remain to NaCl be found that in solutions from 1/32 to 1/8 normal it has a decided acceleration effect on digestion its nancreatic emplace. Up to a normal concentration, NaCl etill accelerates but the , the concentration is increased etill curtur, it created

Using a sycerine extract of natoreas Gritzuer found that the section, even in the arcetest dilutions, hinder the amylolytic ection. A solution of a solution of a solution of the section of the section

Simple (M. SO, Na<sub>2</sub>SJ,) with hite action at a dilution of 1/6400 normal. According to this, Grützuer must have an a very pure preparation. Other workers (to be discussed later) have found sulphates to be in ifferent. This may consider for by the protective action of protein (22) in those preparations which did not show any effect of sulphates.

All acids, in sufficiently dilute solutions, the accele-



. . . retian. For HCl the optimum is about 1/800 normal.

Noteworthy is the state of Grutzn.r that the length of time of heating in making a state of lition effects its di-

Cole (23) male pharmating on the effect of acids and salts on t ( \_ \_ a vlolvtic mutil of dialyzed salivary amplese (tralin). He our' let t'e action vas iron as d by cmell and a so and neutral selection monobasic acide. To action was ecreted by larger amounts of acid (0.0007 to 0.00197 or HCl) and by salts of weak monobasic, dihasic and tribagic acids. Cole was the first man who expressed the opinion that it is wider bry to remove the electrolytes he-Tora t'e errort of added a entrolytes for the investmental. He d the outling ICT concentration to be 1/0000 normal. With both hither and lower course trating the rate of 'glrol, as The ontinum MaCl concentration was N/30. Here the falling off of the velocity or inthe idea did not seem to be so ranger, i.e., in the vicinity of this concentration there was o ren e three t win the WaCl concentration was practically ontimal. "Is point will be further discussed it a later pararand. Ther an optimum concentration of NaCl to present the addition of were small amount of acid (N/7500) lowered the rate of conversion. Te draws the conc. sion that the H ion inhibits and the Cl ion echelerates the reaction.

oreat are not still we not heart to the cut his data as



"" homic point". The work was fore with all possible precautions and controls, it all recent to here agree that for obtaining and the outsit. The formula this method cannot be considered reliable.

A many conclusion which Cale frew from the fact that len optimum connerts for of NaCl was present any added acid inlibile, were that HCl profices its accelerating effect not by neutralizion oltali which may be present. Til ray be a lorical conclusion is all are trust orthy, but that he has not the proper concestion of the source and nature of the alkalinity is my m the following: A preparation i sallvar; mylase was neutralized with HCl and them dialyzed. This, when used in a tion exprisent, show d an acreleration with alled HCL. Since, he claims, there can be no garoxyl ions present in this erest, to ac election due to HCl cannot be brought about by Tecrose in hydroxyl ions. He loed not consider the rossibility of the distoc after of molecules in which sold m is the positive and a protein group the negative radical, and the existence of OH arouse in the protein molecule. A very interesting discurrent trece relations is found in the work of Moore and Fillia (24) on the equilibrium between wrying concentrations of cire an alralies and the protei sof the serm.

Perering to the experiments of Grütznum on the effect of sulphates, Cole states that a found sulphates in concentration even appropriate higher than those used by Grütznum to be enlirely indifferent. Only in very considerable concentration



The exert any effect. The is one of the many examples which are the many examples conditions which bring shout varying results in work of different authors. This urges is to study exhaustively all the factors which is any way influence the ode or rate of action of enzymes.

In 1907 and 1907 a number of articles appeared by Bierry (2524,27,21,27,30) and co-morkers while nie of the first temportance.

These men showed that the apparent starch or maltose splitting activity of pancroatic fuice may be very misleading. Princreatic flice ofteined by testing was strong anylolytic out flewed not after action. To refer the maltase active evident it was congressed fuice hydrolytical meltose quite actively. From this not be any it is seen that there need, ifference in the number or nature of the engmes in extract of tancress and pancreatic juice, but the apparent telefference is simply the to reaction.

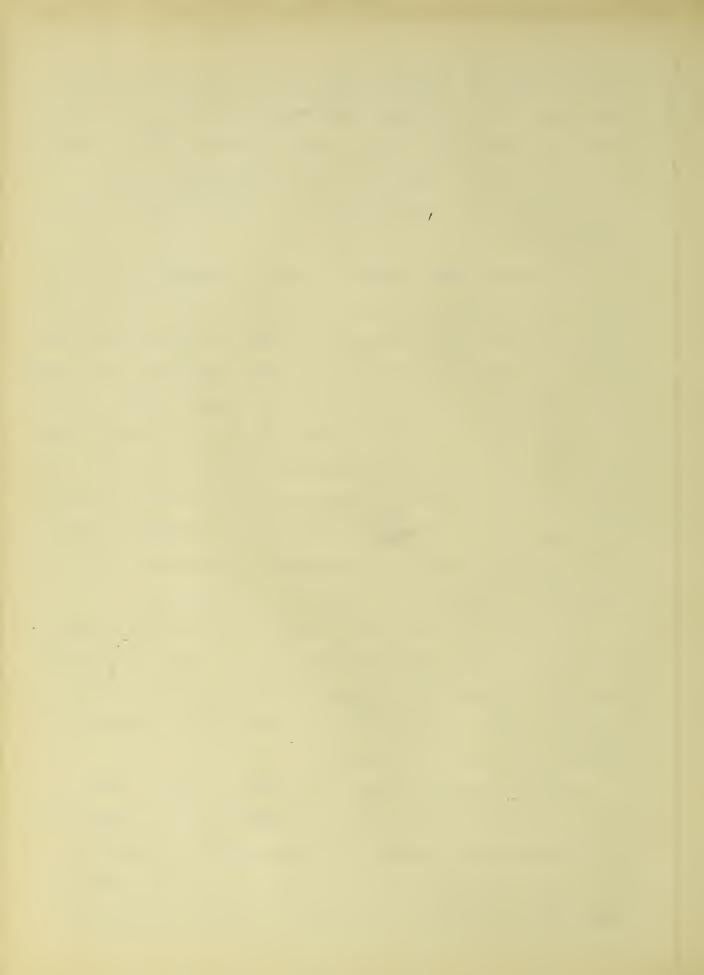
It were not to the more that dialyzed concreatic juice lost its amplicate action. By the addition of acid and calts it may remain both amplicate and maltane action but the addition of increasing a cattle caused the emplace action to become appeared that the maltage.

Pesize inor anic electrolytes certain organic electro
the must be considered. For and Guthrie (3/), who have done

considerable work or starch and its hydrolysis by enzymes,

the sound a peculiar effect of ortain amino acids, especially

aspersoire, or enzymatic hydrolysis of starch. Using starch

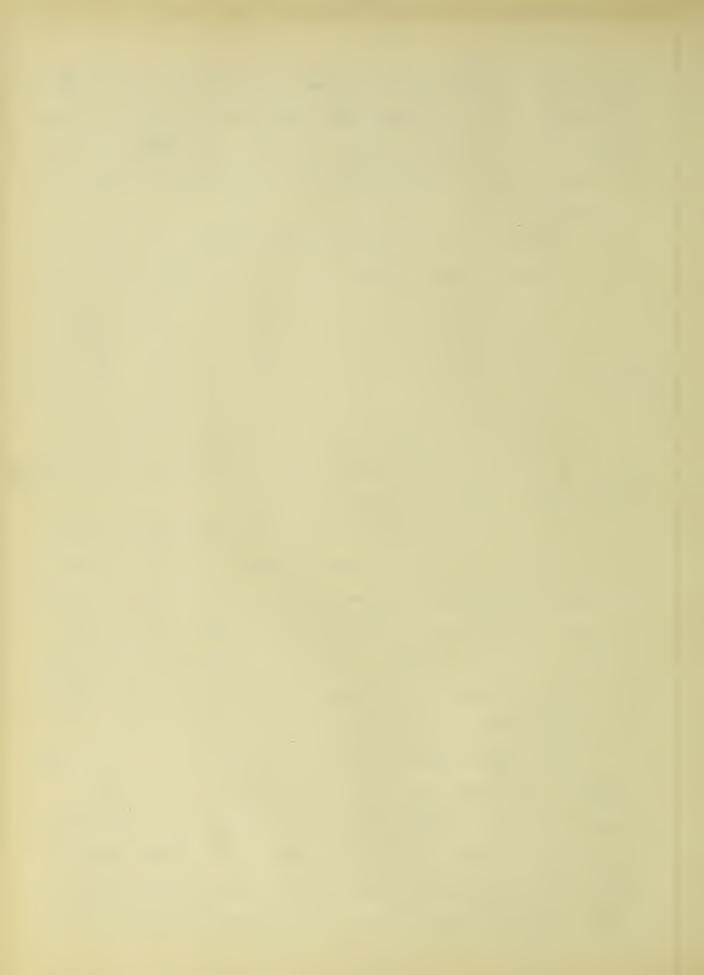


and any lase are an interest of the rate o

Put when starch, not extract, his consider were nine ted were arefully by a consider not only of "freezer out" this marked effect rout in longer he observed.

nation of amplicable action it must be taken into account whether any amino acids or acid amider are resent which could affect the rate of hydrolysis. Then engine of reveral sources are common this is especially intorion. The enzyme preparations are of occurse nature as so that if any amino acids are present me can of eliminate the possibility of such an effect.

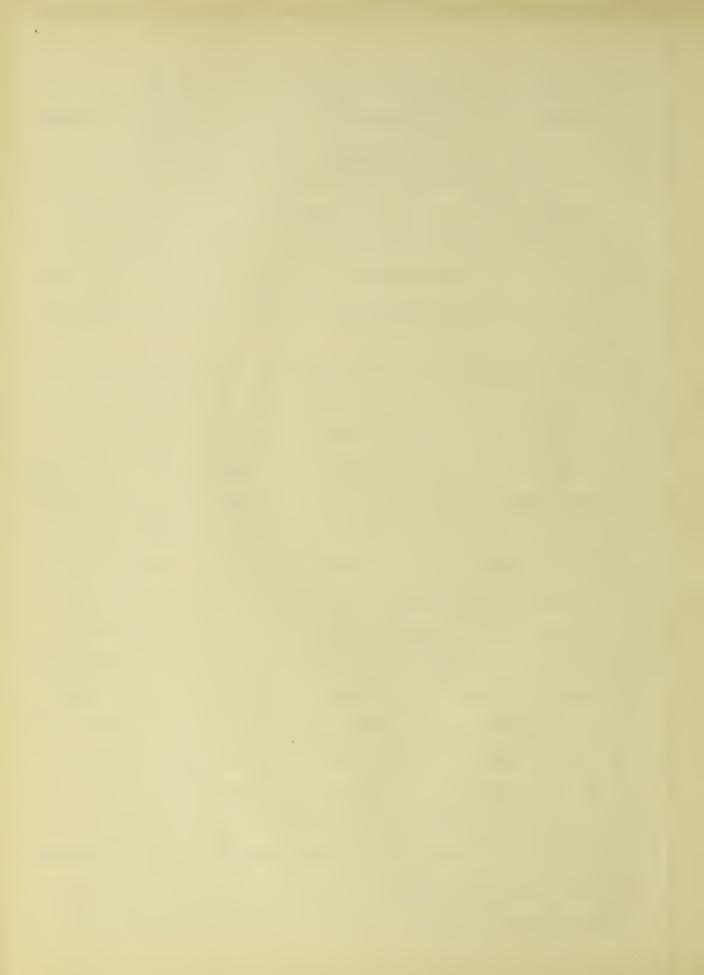
Periode this several other points in the work of Ford and cuth he should be noted. The malt diasters these authors claim the normal mostion to be neutrality which in the plant substitute is brought about by the equilibrium between acid and hasts. Turthermore: "Purified soluble starch has the properties of themely feeble acid; it is capable to the intime.



ions under the real side of standal ositive ones".

France of disease they in it diseases. In the case of diseases they in it did a small quality of mery active of tance of more involved once. This consisted in removing the more in a consisted in removing the more material (Pural distance of the consisted of the filters) and the constitute and reduce pressure. This removing it is a viscous liquid which was the file of distance subjurices.

There ait ore in the early part of their article try to to or entres are cul dir. hit let r the nevert eles, tale this view. I ein orm emidence is that the observed the colloidal particles under the Altra- microroge. This purificant preparation, the authors is a mixture of rall diastases. They wish to have a intinction made enectally letween the state lickefyll and the encountrying enzymes. Tey old rue that on dialyrical to it propagation as a fine water is liquidiying power was not clar ad but its saccharifying power decreased vory manded to. They say that in this way a fairly definite separation of the two groups was effected, the liquefying enzyme havirm receiped in the dialyzer and the saccharifying enzyme diffile of out. They do not report any attempt to demonstrate time e in the dialycate and in the writer's opinion it is quite emident that there was not in like a sen ration of two enzymes That thet the effect noticed was simply due to the removal of erectrolytes by distyris. This is strictly analogous to the



In and the Payliss (33) nives this work of Frankel and Hallur as evidence for an cific liquefying and saccharifyinc engmes.

that there is a difference in the int of the molecules of the liquefying and saccharing on enzymes, those of the latter having nessed through the norms of the membrance. They say that further observations on this noint are in the res, and that our many they will be able to orthin in this way a net of for the actual separation of two enzyme groups. This method has, and that any sared.

Prof.: (34) dialyzed pencrectin solution, urine, blood serum,

--diastage solution and maltin (Merch) solution a single run
rund then are an arised distilled vater. Pancreatin, wrine

and blood serum lost their anylolytic action entirely. In the

attention preparations the action did not disablear entirely

but we weekened. Upon adding 5 cc. of half normal sodium

chloride to the dialyzed preparations those which had lost their

action of the aclt had practically so effect.

The two preparations which were only alim to weakened by



The results evidently to not a receive to each final and farture to each final and farture to each final characters.

Producing the summer of the sum formed as a measure of the sum formed as a measure.

A series of stulies by Wohl-emath intitled "Unterbuchungen inher die Peace et, the first of the populationed in 30, e tell of very certain hie in the story of ary-\_\_\_\_ e. " irst never (36) fare us to e new motion of Wollmeruth which uses as a hold of arm to of ferrent required to cause disappressance of the line of or of a starc's igest in a liver time. The mother is an armoral to an armoral in work in which we must be not the main object and we must bear this in mind in interpreting the results. An alvertage clair wa by "ohla muth for this method is the tot measures the conversion or he have steining starch to lover polysaccherides indepenre the conversion of love the bers of the series. This, he care, rives us urate measure of the power of a ferment to by allyze the starch, leave out of consideration the action on the destrice an oftone. As pointed introduce, this is not this eight the rate of hydrolysis of the stair ferents on the count ty of lext is and maittee or sent and is therefore influerced by the rate of hydrolysis of these lower members.

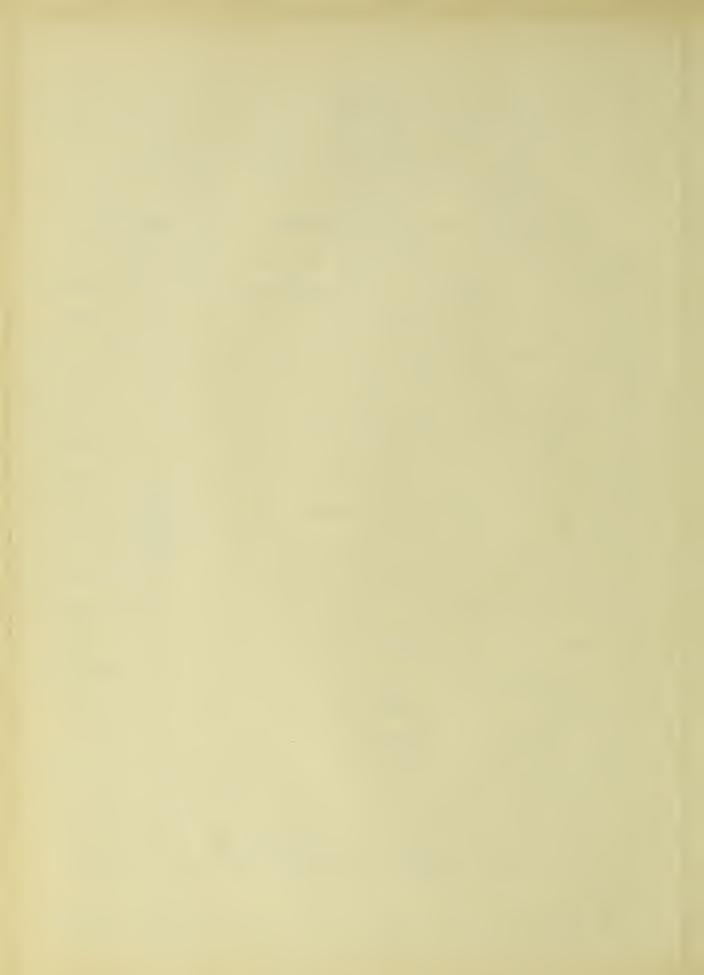
To obtain a method to discretion objection could not be referred, it would be recessary to measure the concentration of atalay and of the decomposition of the with sufficient accuracy to enable one to call the from the rate of hydroly is of



of the lower members of the series that effect on the survey rollysis of the members. The water has a minute vorting out of an employed but and the resulting of the series and the resulting or sucts.

In his around namer (36) Wohlmemith at it is directores of aring and range residently those of solito and pancreas. The firds that the amyland of saliva, mancreas, and blood are identicel in so for a tie efect of acid, la es and lel is concerned. This is contact to the onities of men wo, from -17 corts of -71 + ffer the mode of antion of amylose of merious sources, draw conditions is a line specific anylases. First of all it along striking that refore such conclusions can be italian the nature of the meiting must be taken into coreinerstin. It also recomes emilent that on this basis suffich rt esse tiel p inta of similarity can be demonstrated to outweit by far the evidence of disci Pacity. For instance, Ascoli and Bonfanti (97), or noting that various starches have o verying to of throlysis the clare blood serum, cole to the corclusion the there are crase to the rum specific emylenes; ..., rice sterch and noteto starch. This overfrom concention of moscificity - evidently as to the infinence of the modern development of nothology along the lifes of amounity. That the results of Ascoli and Bonfauti are due to verying resotion and electrolyte content of the staccies is - - - t from Schi nicel', obs reations which were discussed abore.

World it, after author emonstrated in a very striking



the relation of the lattic juice to amyloly the action and conciliate the relation of the lattic juice to amyloly the action and conciliate the nation of the lattice of the HCl man or a time play the part of an ectivator for collings anylone and increase its time enormalist. If may be mentioned here that trutzuum add come to the same concilian long here a Worlsman to the care conciliant long here a Worlsman to the care conciliant where the evidence.

Wo'll repute attributes the activating influence of NaCl to the Cl ion in the case with Cole, but arrives the con-

the of HCl and the inhihit effect of Na CO3 and NaOH.

Sodium prosperte, orally and acet to inhihited; nitrate, retiret, and cross are accessed; substates were without action.

The oranic substances along and leucin inhibited; plycocold

was informate.

The observations or a transfer, on the whole, with those of Cole. With resolute the amino science we must remember the results of Gitties and Ford which show that their action is enried to the six test amounts of imporit.

The monoratic nomes of blood serum be considered to the income of y variable. Before we can seem conclusions as to its "concentration of ammose", it must be shown whether this variation is not supposed that the constitution whether we can at 11 speak of "engy e concentration" or weather the quantitative action is not simply movemed by the other cine of acceleration and institution influences.



Sherman. K mini or C 12 (38) tilled the real in use for Artermin fight of the concrision to the methods in the very important for or has been omitted, .... te octivating influence of latro-The tree wor in the anylase is "Fully activated" and thus it is expected . . letter a ruerent in the results will be obtained. The rathed, for the first, to renormatic emplace. For this engine the fill and intelled and in soom. of NaCl and i . of My 50 Pindim phospirate er 100 cc. (lital r.m.e) of it t cuteiring of coluble starc. The e isportion and in for all necessity at the us. Accordant to Class, as mentioned athre, there is cuite a renumber WaCl concentration which is prectically crtime, but it is a continum whether the point of res\_ often m NeCl concentration i not easily pass ! to. engine preparations of the control to begin with. The results of Sharman are Kerdall (39) seen to show that by roin heyond the ortimm NaCl a depres ion results (Tah'e or name 1099). In most cases this may be negligible but " er the sait content of the enzyme or paration is and vari-hie, it is to be the into collection. Furthermore, in there is uch protein in the engine preparation, the column tions with regard to "free" electrolytes may be note variable. n : at prome in mind if the m to of Sherman and Kennall to have a first apprication.

These altime we so file stare a lifetrate and whire in the copier office.



they correct for the relation of the meanthre hear shown by Clark (40). The reducing nower of the sclubble starch is the not reducing nower of the plan to reducing nower of their own, easily hydrolyzed in the analysis so that the correction is meant. Tenn.

The more of Sherman and Kendell is a very marked forward of in the study of amplases. Some of the more important noints in the raper are the following:

With varying correct fions of salt and a fall the rate

of sycrolymis is a fected but the first bold from tends to be-

This is irretly opposed to the mindings of Cole who work in its salives anglase. They offer he a mossible article the engineer may use the hydrogen and others the hydroxyl ion in her wine about the hydrogen and others the hydroxyl ion measures are actual hydrogen ion concentration.

This would be necessary before drawing any conclusions. It is also write no earlie that other condition, were not the same in the two cases. Just as in the observations of Rosentheler (22) nootein protected the emylase from the destructive effects of ecid and alread, any differences in the quantity of protein or related to the cole as other the effect of pulsaryland and hydroxyl into the ent.

Sherman and Kerdall clso showed that the optimum reaction depends or hold alkali and salt, i.e., optimum NaCl at one con-



representation of all of need not be obtained another and the meaning of the phenomenon of activation and it will require a very intricate tally of all activation and inhibit of actors colore the problem is solved.

Another decided advance in the study of amylaces we firm in the book of Section (###). Instead of the preparation of "organ-powder" and "organ planma". This, if universally abouted, will be a strong of the contradictory remains to the various mathers in which ferment preparations are made. (Glycerine extraction, press-juice, preceipitation with selts, siconal amorphisation, etc.).

Starkenstein used Wold and this method but introduced a modification necessary when preparations rich in protein are used. The liver or an plasmas contain certain proteins which coard are at 37 learnes and in precipitate carry with them a nortion of the starch which this uscapes the action of the engine. This is an interpretation of the tubes are the thermostat.

Ar in ordination of the effect of mothol on the diastatic engine of the liver (Tahilt) momen that almost lift not destroy or matter the engine. Or an plasma (an extremely fine suspender of the order noncer of tained by Wiechowski's method) of a reflict. Tiver was precipitated and allowed to mend with alcohol for neveral hours. I en the precipitate was filtered off, wasted and imposed in physiological solt and ution. The first light and interest of the original



tion. Starte stein thinks it some "some "some "some "some" is hear resource by the sound. Similar that the some "some "s

A second to the end of the pupils, Pial and Hamburger. They find malta e action especially strong in blood and Röhmann (M) admit two possible explanations for this difference between blood and other tissues; either the blood contains an enzyme which acts more energy tically and carries starch hydrolysis to the dextrace stare or there are two enzymes prisent, one corresponding to disset we start the other to "plucale" which so far has been around only in plants. He thinks the latter view more makely on the evidence formished by the experiments of Pial and Hablinger.

Bial (43,44,45) was a e, to a standard precipitation, to the acted on standard and textrins but no longer on melitate. He are to following experiments.

this was allowed to the total for helf of mount to occasional stable.

The precipitate was then quickly filtered off, washed



with alcomplant ether and dried. In resulting power 10 m. were alter to 1000 cc. of l'sterch and incubated for 10 mgs. So for a to a very considerable exhibit. The dimentional was fillered and in the filtrate and an element of the osazone formed. The vas 10.70%. For any maltosazone it is 10.77%.

Practically no lextrole had been formed.

A reprintment experient to a me in which 200 cc. of and 2000 cc. of 94% alcohol were used. The time of experient to the alcohol was assin 50 minutes. Fifty g. of the ferrest cowder were used in this experient with 1000 cc. of 1% starch and the me of indicate on three days. The per cent of the end of the ferrest respectively. From this it is seen that a mixture of maltocazone and dextrospect c was obtained. Maltocazone contains 18.67% of nitrospen.

The experiment differed from the first one in having a larger quantity of the "ferment powder" in the digest. The result in soite of the aborter time of incubation was the production of some dextrose. If there is a specific maltase whose action is destroyed by slephol precipitation, it must have been not a non-equally in both cases. Still, in the second experiment

The result of interpret.

Dismann dis, viz., that moltage we feathward and amylase not. we may but as well, and even more logically, assume
that alcohol was a site of rolyzing activity of the serum on
taken of meltose, and if a millionum mell quantity



etarch more till a conquer out the typolysis of maltose is considered to the typolysis of maltose is considered to the typolysis of maltose is formant bounds. We must remember that the formant bounds are the experiment bases and the activity where the typolysis.

In edydroly-ed eterch with an acid a time could be found in the could be found in the could be found in the corresponding the meltose but very little dextrose was produced. As mentional before, Woll lass such that at certain eteres of the acid by rolysis of starch considerable maltose but very little dextrose is or ent.

Bial did two more experiments of this lind, one showing that 40 m. of the "forment powder" divested for three days with 600 cc. or all maltone solution promoted to appreciable hydroly is in the other that 15 m. of "ferment powder", in the remarkation of which the minut was exposed to the action of along for only for the first was exposed to the action of along for only for the first on of dextruse and hardly any maltone. But Biel an Röhmann cell appealed attention to the fact that the inactivation of the maltane action by alcohol along the along the action of the maltane action by alcohol sector the along the action of the maltane action by alcohol sector the along the action of the sector of the sector of the sector of the action of the sector of the action by alcohol sector the along the along the action of the sector of the action of the sector of the sector of the sector of the action of the sector of the action of the sector of the sector of the action of the sector of the action of the sector of the action of the sector of the sec

rervations made incompatible time, we see that first of all the entraction of the cartion of the hloom string by alcohol is not, the enzyme. It as been mentioned that Stark-



enstein found emplace action of i or point to be increased by alcohol nuclipitation if he ristored the solt look by the trust sent of the alcohol.

The writer has given in the xourment to be described later than an evaporate at cohelic extract of blood serum is strongly amploytic. If the amplase were "killed" by a colol, this alcoholic extract certainly could not low amplolytic action I the case evidently the relation value of salts by the treatment with alcoholic extract the precipitate.

The fact that time of exponent to alcohol has an influence on the acceptant the ferment is explained by the fact
that from the precipited discrete the electrol trising diffuse out
only along and therefore the longer the exposite, the more thoso
only along and therefore the longer the exposite, the more thoso
only along the precipit discrete and acceptant the writer has also sown.

If of interest to retire certain results which

(#6)

The section me of Röhmenn's bundle, a tarned in some further

or on alcohol practicitation of blood serum. In found that

the results which Fiel reported whre true for ox blood but not

"or dor! alood. True time to the lines of blood according to

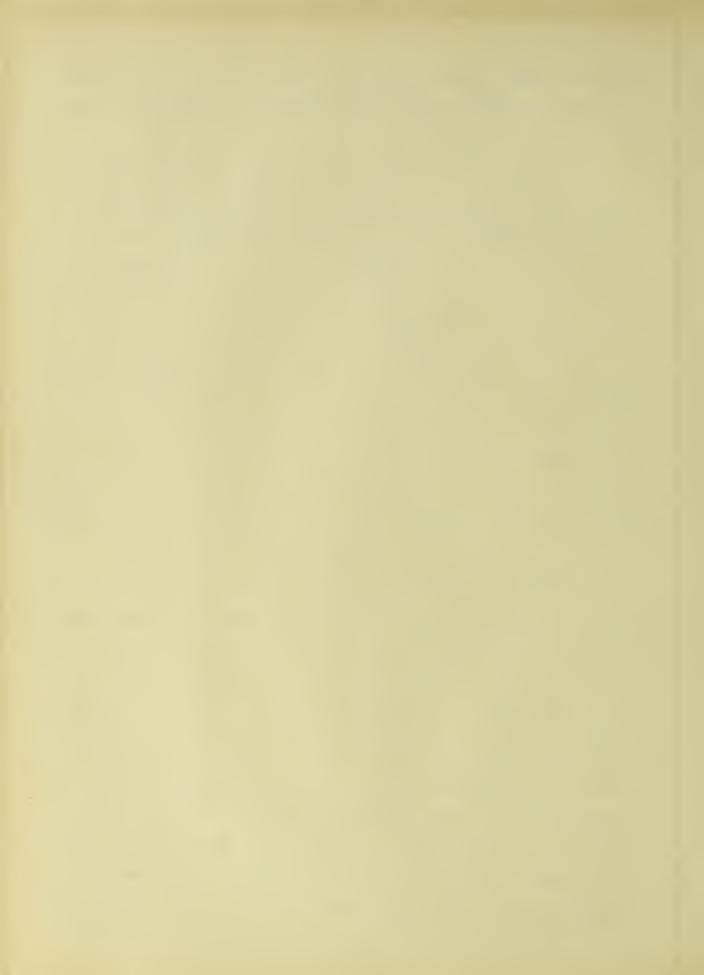
Reflection to the lines of blood to have lost incomplated action but dor! alood retained it. The later from this that

either in every case a partial. Refinite fraction of maltase is

inactivated by a liven true trent with alcohol and that dog!s

blood contains much more that has made as that quite a grautity in the first particular to the distribution of the contains that

it cannot exert containable action, or that there are two mal-



to alcohol while mother is not. Why the blood of the dome mount contain an alcohol resistent maltase, and that of the ox not, he for not explain. To the writer it seems more possible that the blood of two animals may lifter in such substances which mist inhibit or antivate engine action that there are reparate engines are each or tid by the tissues.

Röhmann's of a evidence is insured on Hamburger's (/0) data

the differences of med in the strength of mediane action

of enzy elemenations from various sources. He says: "If the

rete of liquefaction of starch in the increase in the reducing

power are observed, it is fold that these are greater in pan
creatic juice than in caliva and or ater in saliva than in

those serum. Pancreas and saliva produce a rapid increase in

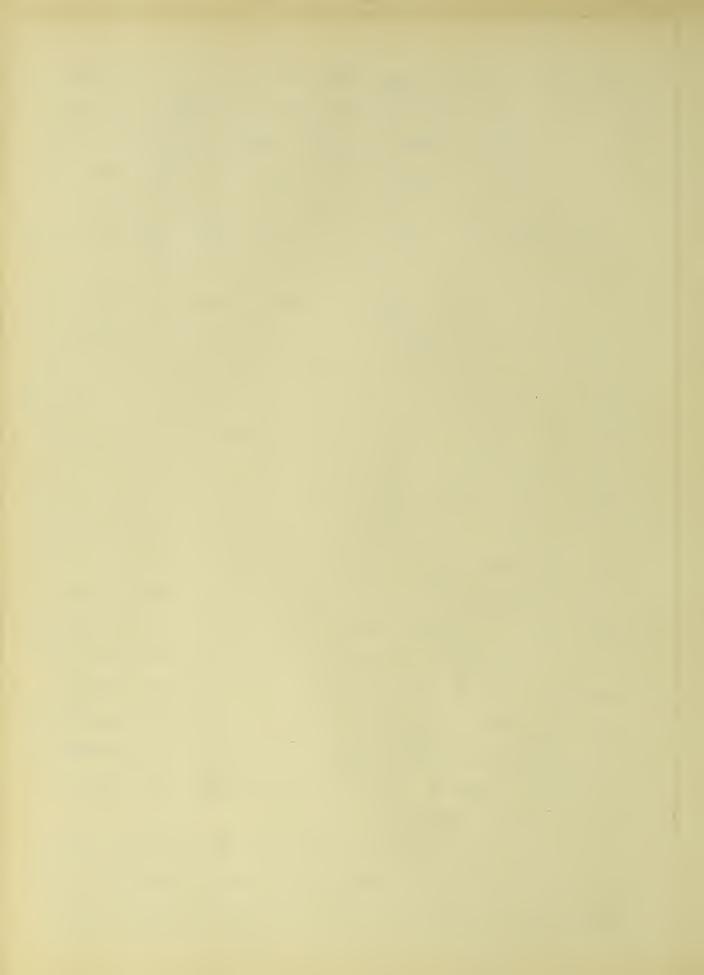
reducing newer until a maximum is reached which is lower than

that with brood serum. This is no theasily explained by assum
irm that pancreatic juice and saliva contain relatively and

ansolutely notes in take (amylase) and blood serum more maltase."

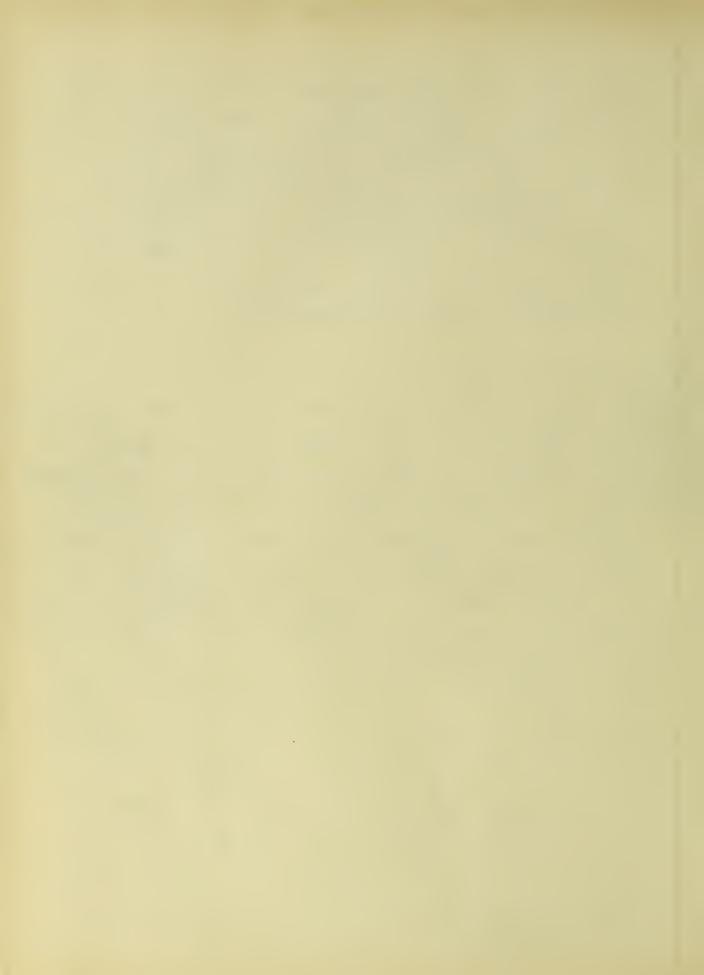
netice or normalization of electrol to a evident. We have seen that maltare action will not do on in a medium as alkaline as rencreatic juice and this may also account for the "absence" of maltare in caliva. Blood is nearly neutral and it causes remidimal ose hydrolysis.

Ore more type of evidence for a specific haltase must be mertioned. According to Puglie to (47), blood serum when slowly helds a sits malter ontion before amylar action isap-



encymes but if he could dilute an acid with successive portions of out it would also lose its "maltare action" confer than the "amylese action", i.e., to appreciably hydrolyze maltose a him is a martion of hydrolyzing arent is required. Besides, as shown by C internal Martin, when protein is heated definite and a in reaction and "free" electrolytes occur. This may also heated on a firm and in Pugliese's work.

The see from the considerations that in consequence of a second or enter the earlier proofs for the existence of a specific and are subject to criticism and the whole question requires representation. There is nothing which fully disprove the existence of maltace, but even if an investigation with the surmose of verifying the doubts here expressed should not accomplish this, the information which it would bring forth remarking the machine of the amylase and maltace action would note it fully worth while.



## PAIT II: EXPURIMENTAL WORK.

## Introduction.

- 1. Theerwations on and Maltage action of Blood Serum.
- 2. Waltare and Althou of Hislyzed Bloom Serum.
- 3. bservations on Bial's "Ferment Powder".
- 4. Detection of Traces of Starch in the Presence of Starch.

  Text in an of Dextrine in the Presence of Starch.
- 5. The Starch-Todine and Destrin-Indiae Reaction.
- 6. Viscosi etric Observations.



## PART II.

in observations on the englese and maltan union of blood corus. As this work progressed it become evident that the lack of proper method course? On deal of uncertainty in some of the results and limit is the men of work. The chief obstacle mental set that it is impossible to determine quantitatively the uncompanion tarch and the decomposition products. An attern was then made to work alt a method for this purpose.

This was undertained but some of the mature of "starch solutions" and their properties. Before oing back to the main problem, it was decided to collect more data on the properties of starch, wire, its viscosity and the influence of small quantities of acids and salts on its liquefaction.

Observations on Maltase of Blood Serum.

To study the meltase action of blood serum the following in the meltase action of blood serum the following in the mere made. An approximately 10 solution of meltose was digested ith marying quantities of blood serum, with home and a mantity of NaCl was the meltase for blood serum of the blood ser

The serm (or low) was generated from the corpuscles by accimentation. During the time require for sedimentation it was rept cool by run and water. The dislyeate was obtained in



the billowing wey: I so a thill far containing 700 cc. of alsetipled water a dializate take containing 50 cm. of blood serum

(some containing to above) were just. This was allowed to some
for two days contained the cool made. The vater was
ther reserved on the old water amagened and boiling water

both. The dializate was continued for two more days and the

dialysate evaporate in the same dish as before. The dialysate

was more up to a millime of 20 cc. While the serum has been a light to lithman, the dialysate was alkaline.

An approximately 1% maltose solution we prepared and 50 co. It not such of flasks. To these were added re-

M. . . . cc. - rlood serum plus cc. of water.

To. 2. ' ce. of blood serum blood. 107 J.CT. cc. weter.

No. 1. 100d serum 113 5 cc. mialysate.

No. 4. 3 co. 1 Blood term olds 7 cc. of water.

Mo. E. 2 cc. of blood and my us 8 cc. of water.

10. C. \_ cc. of 1200% serum hlar 9 cc. \* later.

the color of the total and cubatur at 40.0 legreus. Two co.

of tolors were aided to each first and they were corked with

stoppers lawing a noted on one side to allow exchance of air.

The litter of hyperclassic was measured by the increase in the litter of hyperclassic was measured by the increase in the litter of the litter



lution consider t.

The results are fine to make were removed and the reach power let rmin and the to Munson and Weller.

	5 hrs.	71 1/2 hrs	122 hrs.	101 hrs.	2 weeks.
1	11U.6 mm.	149.2 mm.	168.2 mg.	177.5 mg.	183.3 mm.
◌.	709.7 11	יו וג.בועב	770.1 "	181.2 "	277.5 11
3.	1.7.4 "	207.7 11	144.0 "	183.5 "	15 .2 "
د <u>د</u> ه	_0 "	138.6 "	175.0 11	THO. U	187.1 "
2.	701.8 11	176.7 11	100.0 "	174.0 "	163.2 "
6.	۳ ۵.۰۵۳	113.6 "	155.1 "	172.3 "	181.8 "

The manus which corresponding to complete hydrolysis, when calculated according to the table of the Malker, is 189 mm. At the end of the experiment 5 cc. of No. 1 were my-drolysed with and and the areaction corresponding to 203 mm. of cuprus onice for 10 cc. of the direct.

T ors the following:

- In the earlier stares additional TaCl In the tree additional TaCl In the tree additional TaCl In the tree tree additional TaCl In the tree additi
- two weeks one not allow it to come to one to the level. This



may be at ributed to the alkalingth.

- 5. The soller countities of blub and garoly. E the control of the soll of the
- Let the very interest (in 7, ., for a)

  muly lite the cuartities of lybolyzing agent, the rate

  ly rolyzing should be proportion to its concentration. This

  is swidently not the case. Other influences, either the amount

  of salts on the influences or the result.

Maltase and A Mase in Dialyzel Blood Serum.

I a tall in complete 700 cc. of distilled water a dialyzer tube containing 50 cc. of blood serum was suspended. The serum had been shaken up with a small quantity of tolluland was correctly served with a thin layer of tolubl. The jar was carefully covered and allowed to stand or two days. The water has then remewed and the deligrate evaporated in a water path. The dialysis was continued for the rule days and the second dialycote evaporated in the same with in the first one. The combination of water and made up to 20 cc.

The serum. of the dialysis, had a volue of 75 cc. and was made to 80 cc. with distill I vater.

The implies serum that become alkaline. The alkaling to may have



been the to recterial action, but a community to a construct the content atter more at the bland from the body, even if the most extrere precontions are to en. Measures the hydromen ion concentration by the hydromen electrode method, be into that the rection of the blood immediately after removal from the many is practically trueneutrality; but in a very short time that y recent them are the way of sufficient to be even ent by the use of the more than a paper. But in a dialysate which contains the crystallois in the absence of the mean proteins, alkalinguard be expected to here e evi ent in accordance with the views of Moore and Birland of the equilibrium between acids and alkalics and the proteins of the serum.

To study the effect of the dial, set serum alone and the dialyzed serum in an an implent quantity of dialysate added, the following time tion experiment was made.

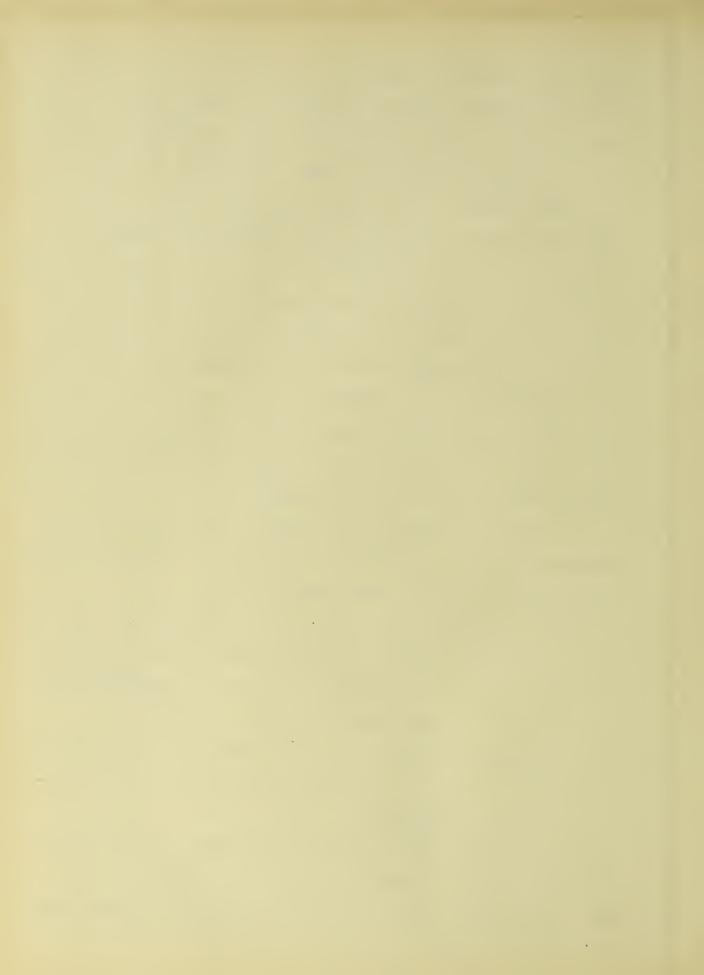
For makes were asen, containing respectively:

- No. 1. 100 cc. of approximately 1% starch paste

  (arrownot) plus 20 cc. of ialyzod serum

  plus 5 cc. of water.
- o. c. and cc. a charch parte (same as do. b) plus

  dc. of di
  al./sat..
- No. 3. 100 cc. of 1% maltose and tion plus 0 cc.
- 10. 4. 100 cs. of 1% remore (the es above) plus



The second of th

The first state in the solution of the state of the state

Time.	7	۵	3	4
O hrg.			25.4	26.1
18 h.s.	Principally starch; a little dex-trin	To starch Erythro dextrin	14.9	19.3
40 hrs.	Marked state st. Con- siderable lextin	No starch V rv ittle dextrin	lost	18.2
¿೧ .∃∈ .	Considerable lection lex- trip test.	No stare . Trae of	1	17.2

This experiment in the node withy fact that alding an entire entire to of the three to the dialyzed serum in-

The mineral of the strategy of this on the strate to



evidence end = manced, if it merchot to a tacts room to a sed solet now, which were not so in Prot I.

The contract of a sed solet now, which were not solet in Prot I.

Sideraul, and the present and quantities of the present and quantities of the meaning the present and quantities of the meaning the present that the present the present that the present the present that the pres

Si prives that in a prijeris of maltose l te effect of he and the less marked than in the case of can must. He surrests as an explanation that prof to the contract of core and ship in all which is made tose, any added selt word include the illowanty of come gunar more than it would that it malto be. Therefore, all other things being and, he ment of a nutral salt on the mobility of hydrog n in, which is the cotelytic a ent, would be greatur in the case of came wher than of altose. In the effect of the and ed tance is on the of the and not on the catalyzer. This conception we among to our experiment and the interpretotion would be. Moltose is wery if icult to hyprolyze er amatically in an almo we madium. Starch is so much influenced by or d by neutral calta. The lat te pentral line and rich in sales, take the importance of the property that the starch. The effect then is orn lipally of cubstrate and not or the engy e and this type of ixperiment will not ledie the lestin until hore data ave been collected on t e effect of adde of mes on the rate of ydrolysis of surars with relycaconsmitter in the one of the contraction in - of hield in all of circles in excluded, as hydren hield and

colloi ol metala.



"Ferment nowier", as used by Pial, Awas prepared as oflows:

100 . of the (ox blood), which has been thereon by separated the corpuscion of a cohol (95%) and allowed to ethal for 30 minutes. This was quickly diltered through has used paper by suction until the filtrate was alear. The precipitate was spread out on a class plate. When the it was a slimbtly color d, brittle publishance when firely ground up in a mortar. Fifteen ground powder were obtained.

pressure of about 100 mm. Intil its volume has been reducted to about 10 cc. In this way the alcohol was all driven off. It was made up to a molume of 40.0 cc. and each with toluble.

Ar experiment was performed to study the direction of stanch by the "remount powder", as compared with untreated serum and the lower to which a corresponding quantity of the alcomplic extract had been added. The directions were done in 3

- I. CO cc. 1% as went starch who file form. ferrent number. (The reaction, after mixing, was neutral to lithus.)
- II. 200 cc. 1% acrowront starch of 19 to cc. of blood serum

  ( and sample as used for proparation of powder). (The
  reaction, after mixim, was neutral to 11 mis.)
- III. 200 cc. 19 arrowrunt tarch plus 3.75 cm. "fermert



cuantities are neutral to lithe).

Time of tolucity.

## After 24 Mours

- I. Glucosathre and maltos zone test negative.

  Game less reduction with Fehlin, 's solution than III
- zone ' ' ('icroscopic exami ati)n).
- III. Glucosarone and maltosarone tist negative.

  Order one reduction wit Resignate solution Family.

The reduction test was carried out as follows: 5 cc. of the limit were nearly doubt and ? Props of HNO3 and to compare the protein on warming; 5 cc. of cold water were then acces; Then of the filtrate were then reasonal out and 2 cc. each of Feblina's CuSO, and MaCH was added. The tubes were then dinner into a bulling water but and the rate of decoloriation objected.

Adding the figure of the directs showed extrine in II but in I and III no directly his noticeable.

A line direct

- I. Clucosasu e in Planazone tart nemative.
- II. Glucosazore test strong positive.
- TIT. Crice are en' maltocaza i tost nemative.



So ar that als show that the serum rapidly formed dextross. The two linests is a not get a good any faltone but
the hydrolysis was slid by oreater in III than in I, as shown
by the strong test.

From I and III 10 cc. were now remoted to evaporated to a small bulk in Phillip's leakers. Then, WO cc. of 95% alcohol were allow to and the precipitate was allowed to settle the following the man and the precipitate was allowed to settle the following to the reducing power was then attended in 25 cc. of each. The other 25 cc. were was then attended in 25 cc. of each. The other 25 cc. were the colors of the colors of the colors of power than attended in 25 cc. of each. The other 25 cc. were

T. J. 1 - 1 - 2011 . 0272 mm. CunO.

Ratio: 7,42

111. If setly: .000 mm. Cus0

III. 'y'ro'y mod: .0381 mm. Cu20

Ratio: 1.96

ressed further. Although the addition of the alcoholic extent in ant restore altase action, it causes a mreater production of the alcoholic extent in ant restore altase action, it causes a mreater production of the series starch to dextrose.

A ter days.:

Twenty-rare cc. portions of I and III were removed and trate a larger.

The realt ore

I. Pirectly. - .0074 m-. III. Firectly -- .007 mg.

I. hydrolyzed. . . 1080 " III. hydrolyzed. - . 1080 "

Ratio 1.96

Patio 1.78

Amin Town callo was obtained in III.

With o'ne I slowed reillier - roh nor dextrins; III

Art o deva.

At this time I show no colorable nextrins; III gave erythro ertrin t st.

Twenty-live co. of each treated as histore wave:

I. direct J. -- .0 Mg. III. irectly -- .0673

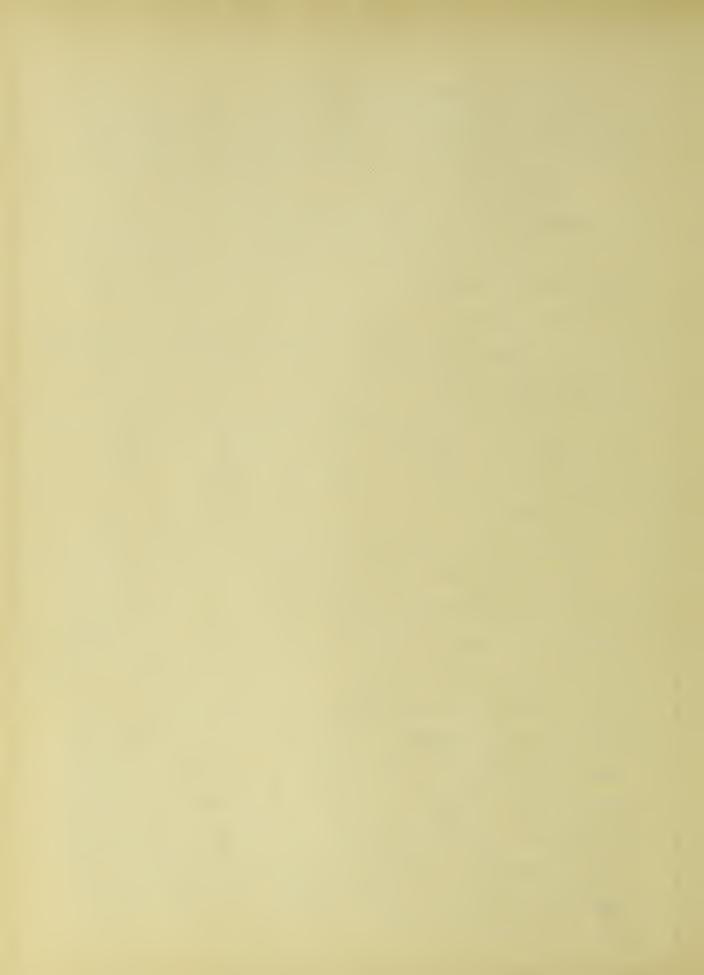
T. hydrolyma. - .1064 " III. hydrolyzed. - .1396

Ratio 2.49

Ratio 2.23

Result is the same as before: more dimestion in III.

The rights show that although maltase action cannot be rectured to the "ferment powder" by adding a corresponding a curt of the child extract containing some of the salts removed, the action on the stand is slightly increased. Chan as brought about by the tree ment of serum with alcohol are so profound in both "powder" and extract so that we cannot say that the treatment has been equivalent to removal of salts. In a profound in both series on the colloidal state of the enzyme and this, of course, is modified a maiderably by the coagulation it alcohol. A study of the colloidal nature of the enzyme and the factors influencing it, the critical hopes to take up in the near future.



Another exp riment on the "ferment powder" of Bial was perform i.

I and III were the are as lefore.

II. 300 cc. 7 starch plus 10 cc. alcoholic extract.

By iodine test.

I. Starch, no mextrins.

I . Show on tent in thtful, \_\_\_\_\_\_ro de tries.

Til. Tittle to de, mortar dextrin.

conference "canylage action. The fact that II was digested so vicory and shows that a cohol does not destroy the enzyme.

The fact that II was digested so vicory and shows that a cohol does not destroy the enzyme.

The fact that II was digested so vicory and the enzyme.

The fact that II was digested so vicory the enzyme.

The fact that II was digested so vicory the enzyme.

The fact that II was digested so vicory the enzyme.

After 24 hours.

Same results as a too Louis. All diges is had pro-

in III that in I.

I. directly -- .0265

III. 1 cot1y -- .0747

III. hydrolyzed -- .1599

Ratio 2.50

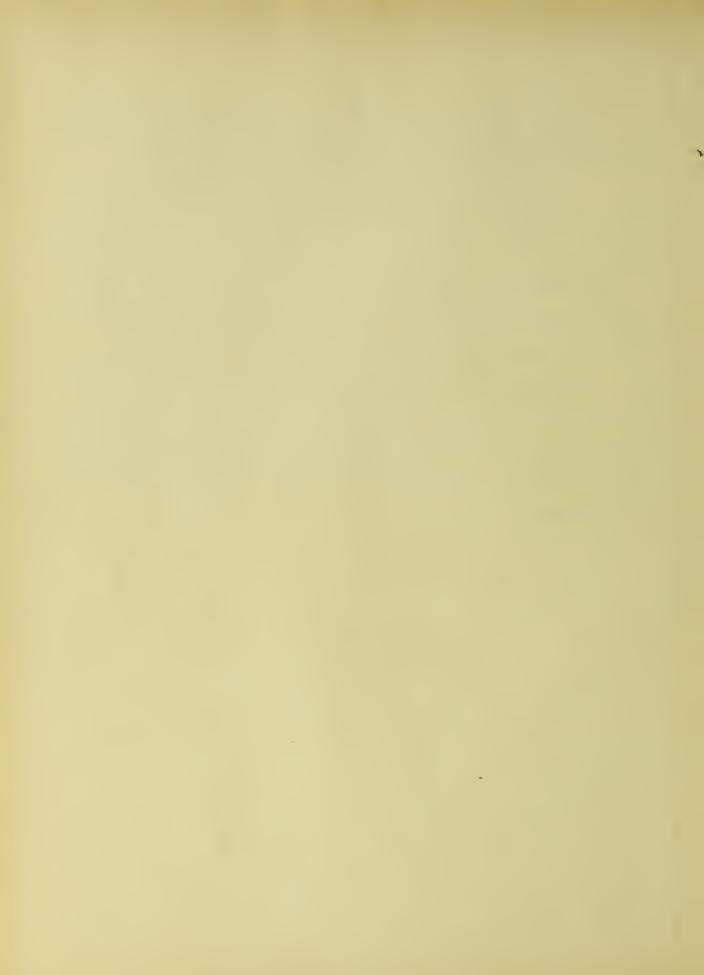
Ratio 2.14



are never new and was on the ear albumen and fouch.

added. A the 20 minutes in the inpublic at 40° the mixture was trated for dextring by adding iodine to slart excess and then ecolorizing with MaOH solution. The test was positive. Immediately after a king, no dextrin was detected. After two hours the first is in the digest were muite noticeable.

Why show he ear albumen ou tain an enzyme specifically shows only a very main notion but it was amylase action. Firstles work has been controlled to stronger action.



Preserve of saces of the Preserve of Saces of the

in the presence of textrin which ordinally obscures the blue color. If a solution of extrin, (as ourchased for our dextrin,) and a red color is the first in the artist in the artist in the artist of the artist, (as ourchased for our dextrin,) and are are the mith loding in the artist way, a red color is obtained. But if a later of the artist solution is superimposed upon a factor of the solution of NaCl containing some indicates the corner at the surface between the two layers. This indicates the process blue staining to a deep blue are are the surface between the two layers. This indicates the process blue staining the color is usually mealed by the large expant of red staining dextrin.

Ar and instion of this total was more in the digestion experiod of ith blood secur. All the digests of blood secur
and stand (green after two werean incorporation at 40 decrees;
tolded as anticeptic), when trated in this total area blue ring,
although, by testing in the ordinary way, they stold only the
left of or. In the cases free this was very weak.

I stand is modificated with a slight grees of indice of a solution. NaOH (solution of a solution) is conton—

1 and the character of the color will stale.



it is noticed that just as the house color is an earn, a refinite as earn as much the terch ordinary, does not have entred in an earn which the terch ordinary, does not have entred in a color with a line. When the arrowant starch used by the will, the body are of much in concentrated starch and color of the actually faint, take with the notate starch and several starches on the matter, whose orders was not determined, the total extranely faint, whose orders was not determined, the total extranely faint.

This should that the arrowroot starch used was practically free from dextrin, while the other starches contained quite roliceable amounts.

The Starch Iodine and Dextrin Iodine Reaction.

The starch is ine reaction which even the elementary student of chemistry is so familiar with, has, in its principle, never been explained. A very plausible explanation has recently been offered by Harrison and defended by Estwald on a theoretical rapis. But the explanation by Estwald on a theoretical rapis. But the explanation of the process as an alsorption phenomen. The color shown is, it willoidal indine that the process as an alsorption of the polysaccharine present, and depend on the degree of dispersion of the polysaccharine. Ostwald shows that this in in hermal, that the rule for relation between color and the polysaccharine of the polysaccharine of the polysaccharine.

In the older literature (54,57,58,59,60,61) a moral nany ral-



" seers to lit the onse an "ell as Harri , 's.

As mentions harme, the fact that the "stand in the comround" is asily no mitator of to an attend to securate the
starch in the law.

## Experiment .

A moll ountity of state of one to treated with Ip in KI and a few one of N/1 HCl were added. The blue compound precipitated and was filtered of . The literate was boiled for one hour with HCl (3 cc. for 100 cc. of solution) and tested with Febling's solution. To rejuction was found. The starch hall the producted oughtitatively. (This was verifical repeated-ly.)

A large number of experiments were then done to determine the heat proportion of  ${\rm I}_2$  and to be used for complete procipitation of the starm.

## Exp rir ont 2.

Mert separation of starch and ionine was attempted. A 1 description was prepared (Merck C.P.). This have a polariscope realing of 3.20 in a 2 dec tube. To 30 cc. of this extrin 5 cc. of 1 starch botte, 4 cc. of  $I_2$  (in KI) solution (approx. 1/5) and 1 cc. of 25%  $H_2SO_4$  were added and the volume was brought to 60 cc. (Twice the volume of the dextrin).

After chaking and ellowing to stand for a few minutes, this was nittered; 20 cc. of the illtrate were then extractally toluble to remove I. (This, by this), in the lower country of the interest of the inter



The rotation of the ecolorized solution was bound to be

Fifteer cc. this ware by Irolyzed with 1 cc. 25% H2Sc4.

A control experiment in which fextrin was diluted with as water to correspond to the other treatment mave, the rotation of dextrin

A r hydrolysis (as lore)

This rows that the extrin had been quantitatively separated from the starch. The data after hyprolysis eliminate the possibility of balancing of errors by lost of dextrin and cain of starch.

A number of experiments of this kind were carried out.

In all these soliven amount of stand marte, (arrowroot), dex
trin C.P. (Kailloaum), office and dextrode were mixed to rep
room to a first hydrolyzed. Fairly nod results for the sepa
rotion of stanch and lextrin were obtained.

That this method was tried for the separation of the

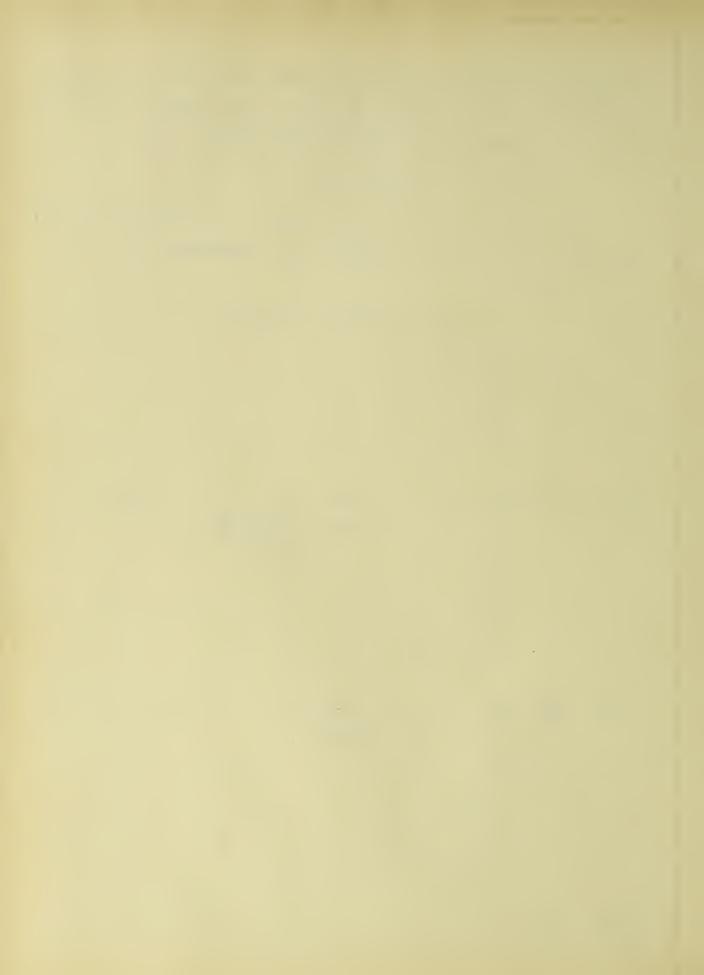


turneting facts turn due, which is owned to that it we used ess to further attempt to one out a method on this basis. They are well in astrated by the following experiment.

## Exp riment 3.

Four sets of int of tuber each were to ave as Tollows:

- I. Each ture: 10 cc. 1% soluble tarnh (Kahlbaum) plus 1 cc.
  - (a) 2.0 cc. N/10 I 2 solution.
  - (h) 7.5 11 11 11 11
  - (c) 1.0 " " "
  - (8) 0.5 11 11 11
  - (e) 0.2 " " " "
- II. Far tabe: 10 cc. 1 \* terch plus 1 cc. 10 % H2SO4.
  - (a). 2.0 cc. N/10 I, solution.
  - (h). 7.5 II II II II
  - (a) 1.0 H H H
  - (a) 0.5 " " " "
  - (e) 0,2 " " " "
- III. Each tube: 10 cc. 1, starch plus 20 cc. N/10 I2 solution.
  - (a). 1 cc. 20 NaCl
  - (h). 2 11 11 11
  - (c) 5 " " "
  - (2) 7 11 11 11
  - (e) 10 " " "



- IV. End tube: O cc. 1% starch plur 20 cc. N/10 I, dition.
  - (a). 1 cc. 10% H2SO4
  - (6). 11 11 11 11
  - (c). 5 " " "
  - (3) 7 11 11 11
  - (e) 10 " "

Results: After 14 noing stending, sets I and III and II respectively and IV, tere prooficelly alike.

In sets I and II all tibs showed a sli . blue precipitate in the bulk of the blue stain of pubstance did not precipitate out. There as no clear supernatant limit in any of the tubes. The color varied from a purplish blue in the "a" tibes to a clear blue in the "e" tubes. The opposity and apparent wind ity in early successively from "a" to "e". The two "e" to be closed a clear blue solution. Two extra tubes were prepared just like those in II, containing 0.1 cc. and 0.05 cc. of N/10 I, colution, respectively. These also showed a clear blue wid here elicitar as the concentration of I2 decreased.

In sets III and IV.

- (a) more, replish plu , very rist sediment.
- (-) " " " " " "; ap-
- (c) -cle superrotare liquid; all blue staining material precipitate.
- ( ) like (c); supernate t liquid a little limiter than in (c).



(e) - live (c); apen en' liquid e lit la li hter tran (d).

The store that is the process of the dextrans contained in columbia teach, the of so long precipitate as before. This is attributed to the stabilization of the dextrine or the curin-ioline complete.

The experiment was no time with set II in the pullowing manner:

The live of this were little to the largened filter.

The live of "b" blue meterial went through the filter.

The live of "c", "d" and "e" filtered clear and all blue materated on the filter. The precipitates were vashed with indice and NaCl solution in the same of ortion at used for precipitation.

The m thor used to remain the starch in as ourse a form as nearly le was the follows: The precipitate was warned off the filter paper into a flask and treat of the Ha2S23 to remove the indirector the complex. The starch thus we thinto solution and was then precipitated out with Ba(OH)2 solution. The precipitate was militered off, washed into another flask and treated with Ma2CO3. The precipitate of BaCO3 has then filtered one and the solution tested with indine. On decolorizing with NaCH, as described a removed dextrin test was obtained.

Trom the remarks and many other similar ones the



- of all processing the compound by the compound of electrolytes.
- The lext is that occur in soluble et meh, with trusted or descriped above, are notically precipitated and may have a "starting action" on the colloided "starch-iodine".
- 4. The implication for the above is that when starch is nartially hydrolysed, a select of dextrine is formed. In the nurified preparations the higher numbers of this series are removed. Here higher sembers have as "lobe compound" very marking ansoid preparation of the starce.



## Visco imetric Observation.

chemical function of annual number of Ferral Landingstons have for an impact work on the moint but their work is lifficant of interestation. Manually and Roux (62) have come to the concention that starch grains contain a mixture of two substances which they call anylong in anylongetin. Anylongetin the arrival and interesting in two variable; the land anylong in the landing and engages, yielding meltone only by rolyzed by both acids and engages, yielding meltone only. A ylong tin, on the attentional, is comparatively recipted to hydrolyzed and engages them interesting dextrinations. About 80-25% of the starch is anylone; 15-50/ is anylonectin.

Macuer e' of preparation is andicated and the intermediation of his work is call. (Hammersten in his Lord of his work is a call. (Hammersten in his Lord of his work is agreed and the intermediate of his work is agreed to the Rose of the Arbeiten er menanten Turcher richtig verstanden hat, ..... etc.)

Four (63) and also Getin-Gruzewska (64) is subjust for the prenous inn of a "-dome struch" which Maquemme (66) pro-nounces identical with his "amylose". The directions given by hith Four of Calin Gruzewska are very brief and uncatisfications. The writer has tried to obtain an amylose preparation by him time that has, in fir, it been successful.

Without do bt, pure no man tions of any lose of anylopectin



action. Since the material were, or int, not avail-lic, we have not and "solution" of Kahiba m Soluble
starc.

Fourth which the obtained by collocion diltration, has in the collition (not a colloid). As ordinary he have the following data on the viscontrol this state compared with water, case sugar, and colloid that he sill tion.

hardly wree that of the "Time diamentatel". Bottazzi (3) and d'Incape that of the Time diamentatel". Bottazzi (3) and d'Incape that the 2 minutes, 8 3/5 seconds, while that of vater was a minutes, 4 4/5 seconds. This makes it extracely that the "true solution of starch" was a sustenced. In we assume this, it will help us in understanding the reality here were on the viccosity of starch.

or two colloids modifications of the ame polysaccharide. The amplications of the ame polysaccharide. The amplication is a suspensoid, the mylopectin an emulsoid. The amylose in the standard loes not show the usual properties of suspensois ( .e., precipitation h. all quantities of the



the lates) on account of "the mulsoid of the emulsoid of all orders. The conference of all orders in the presence of an emulsoid, either a recommendation of the conference of an emulsoid, either a recommendation of the conference of an emulsoid, either a recommendation of the conference of an emulsoid, either a recommendation of the conference of an emulsoid of the conference of the conf

Ferrhoov on Tolf have studied the mechanism of the liquefiction of starch. The lique action should correspond to

by relycia. As a manner of the lique action the use the relative viscocity, as indicated by the time of flow from a buretue.

The effect of monobacic and distance solidar receptants of sodium hydrotice and the submater of conclum and magnesium on the rate of liquidation of starce parts of the perature and measure are turied. The monotonic transport than the normal alkaline starce.

Are any basic salts depress the lique action; and salts accelerate it. The CLSC and IgSO have no effect.

In connection with the stady of the viscosity of starch. The time of flow through a capillary tube attached to a 25 cc. pipette bulb is given in seconds. Fifty in nortions of 4% arrowroot starch maste were autoclassed for approximately 35 minutes. The temperature was 120 parces.

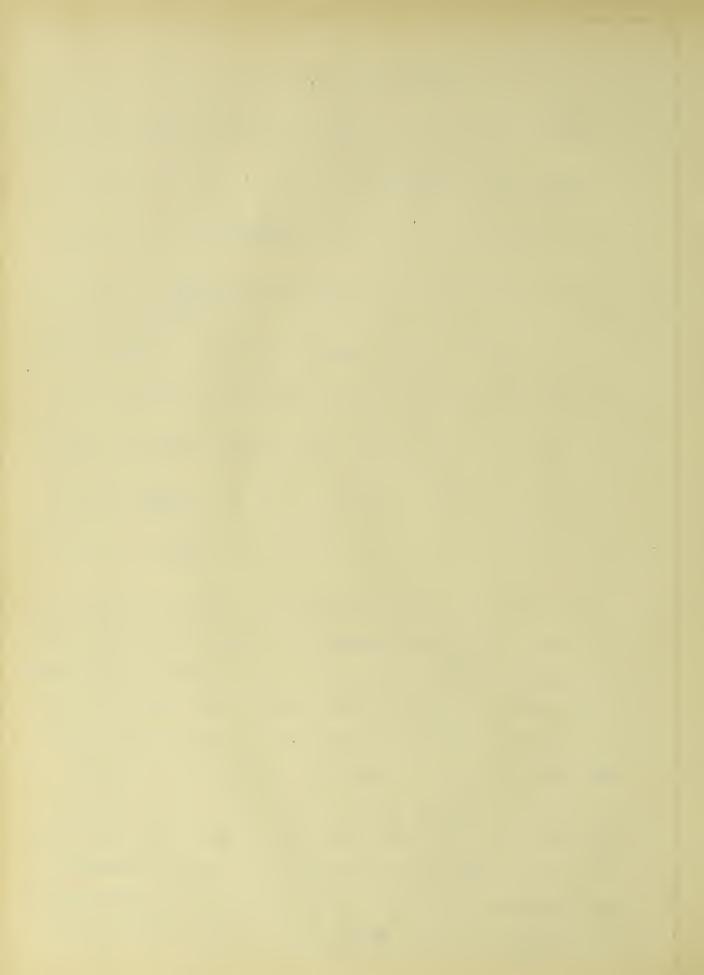
The starc' (arrowroot) mave an alkaling reaction with contact. To neutral the FD mm. of the 4% paste there were required 1.0 co. of N/D sulphuric act.



## Experi t 1.

Woter			£C3.
Starch,	not mut oli	-78°154 °	1
11	H H	pl : 7 cc. 1/5 mol.	11
tt	11	plue 1 cc. 1/5 mol. a <sub>2</sub> HPO <sub>4</sub> 290 "	9
11	п	plus 1 cm. 1/5 mol. NaCl. 249	9
88	neutralized	$=$ (1.5 cc. N/. $\pi_2$ S $\pi_4$ required) $=$ '	t
11	11	pl _ cc. 1/5 mol. NaH PO 50	H
ŧŧ	11	и и и и и Na <sub>2</sub> HPO <sub>4</sub> 225 '	19
81	11	и и и и и NaCl 23 <sup>в</sup>	89

This was and of the preliminary experiments and loes not claim any high degree of accuracy. In general, it agrees with the results of Pernback and Wolff. The difference of the effect of the alts on neutrolized and unneutrolized starch is strikting. Sodium chloride has practically no effect on the neutralized starch but given a much higher figure when it is not neutrolized. This, as will be about in one of the following experiments, if not much to a difference in the amount of hydrical mut is a compulation of the suspense of component of the aterm. The effect of the point the war also different and the left of the point of the starch. (Further distance the starch is a contributed to unneutralized starch. (Further distance the starch is a contributed to unneutralized starch.



cos in o to on'er Experiment 4.

Exp rimento with the effect of acid and salt on the list of sterch and its rate of liquefaction. The office the observed immediately, after bour: standing, and after howing the solutions in a boiling list (98.2 legrees) for 10 minutes.

A wincosimeter of the (the type was used. The till for the flow of the rest of the contact of the egrees, using a representation resistering the first transfer of the second.

tion of used. Into 6 manustra masks (100 cm) the amounts of HCl and NaCl liner below were about dout. Then 50 cc. of the standard manuscraphic simply 50 cc. of sward made up to up to 100 cc. Time of low is given in leconds.

Experiment 2.

			· · · · · · · · · · · · · · · · · · ·	<del> </del>	1
	Cr. of	cc. of N/l haCl	t immediately	t slor 15 hrs.	after 10 min. at 98.8°
٦.	5	10 AS	110.4	109.0	92.5
2.	10	eno Std	110.0	110.0	90.2
3.	an en	10	111.0	109.8	109.0
4.	min first	20	110.8	110.4	110.0
5.	5	20	111.0	111.0	95.3
6.	5	20	111.	111.5	91.0
С год			113.1	114.0	



Txp rument 3.

	cc. of	cc. of	t	t after ld n rs standin r	t after 45 in. at Je.8°
ī.	5	2	94.4	95.1	47.4
2.	5	5	95.2	94.8	17.C
3.	5	10	96.2	94.3	47.8
4.	2	2	9.7.9	93.4	54.4
5.	2	5	95.6	94.2	53.8
€.	2	10	95.4	95.2	50.5
Control			102.0	101.0	

The addition of either solt or acid lowers the viscosity

Without heating, there is very little difference between the effect of the acid and the multiple quantities used.

Without acid, the boiling with salt for 10 minutes does not affect the viscosity appreciably.

In the orange of acid the salt has the effect of slingtly reising the viscosity.



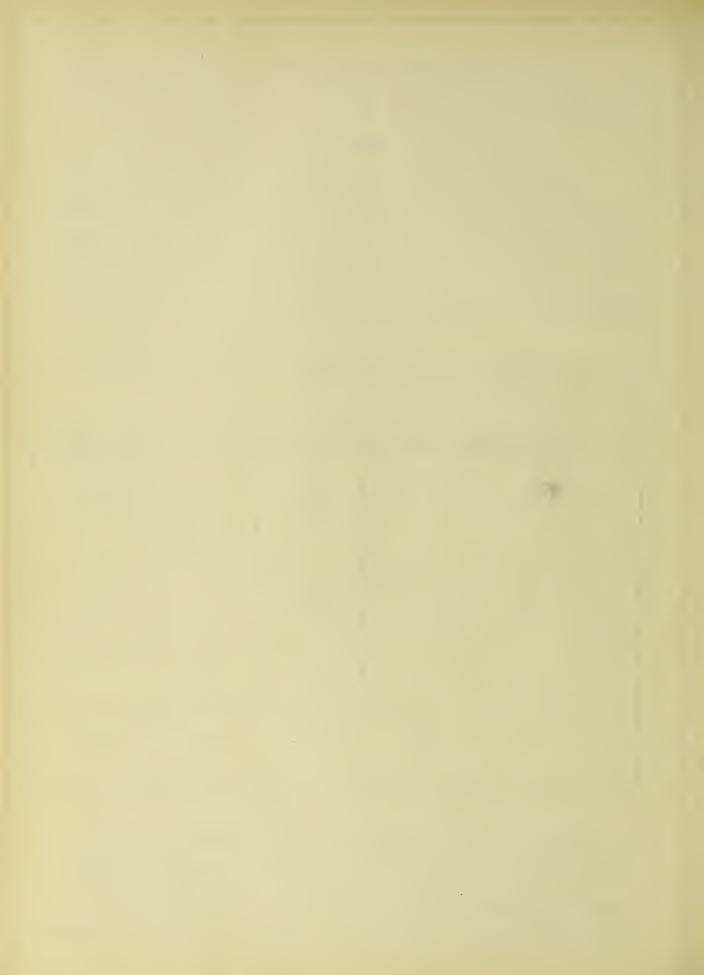
A furth represent was cone with a interconcentration of ctarch.

## Exm riet /.

Fifty ... of a 20° relation were well ned out into each of fire irs. The miven amounts of FCl on NaCl we under out the relative were kept in a bath at 98.3 Terrees for 30 minute and then rapilly cooled by immersing in a mixture of ground ice and water.

	cc. of	cc. of	t er 30 min. at 98.8 Perrees		8.8 Decrees.
٠.	5	0	I 90.4 90.5	91.9 91.0 95.2 94.2	111 90.0
2.	=	10	97.4 97.4		
3.	10	25	1 <b>0</b> 4.5	105.8	
4.	5	40	104.8	107,2 112.4	105.2

Column I im a market of a interiately after running the solution into the viscosimeter; Column II after solution had stood in vincosimeter for a variable limith of time (S-3 minutes); Column III, reading obtained after shak-



in up for a livele Wille.

The most the rate of the list of starch is 0.05 normal HCl as Arrhenium (2/) is owned in the order of the surar. Bosides the discosity meralments, the color liven by the partially hydrolyzed starch with indine was observed. Several concentrations of both starch and in the wore used but in no case could any differences be detected in the state of color (a mixture of blue and the entirodextrin red). This seems to show that there were difference whether in the rate of hydrolysis in the resence of varying quantities of Mail. With 10% cone curer in the presence of C.Co normal MCl. O.4 normal NaCl, Ar therital observed by rolysis 25% creater than with the acid alone.

stancy of those in I. Coarulate particles (of the suspensoid component) will sattle of the bottom of the large bulk of the viscosimeter if my the satisfies allowed for settling. This lower bulk of the solution is the one drawn into the small bulk for masurement of the time of flow. Thus, it may settling a permitting, the verse of the time of flow. Thus, it may settling cannot be seen but it becomes when the bigh. This settling cannot be seen but it becomes when the shaking the viscosic meter, when the verse of the practical the original ones.

The explored variable to the measurements in case there are used by consulction. The writer also thinks that in the coard and conserved at is prosent there is a shart coard at tion so that, as in column I, a higher viscosity does not mean



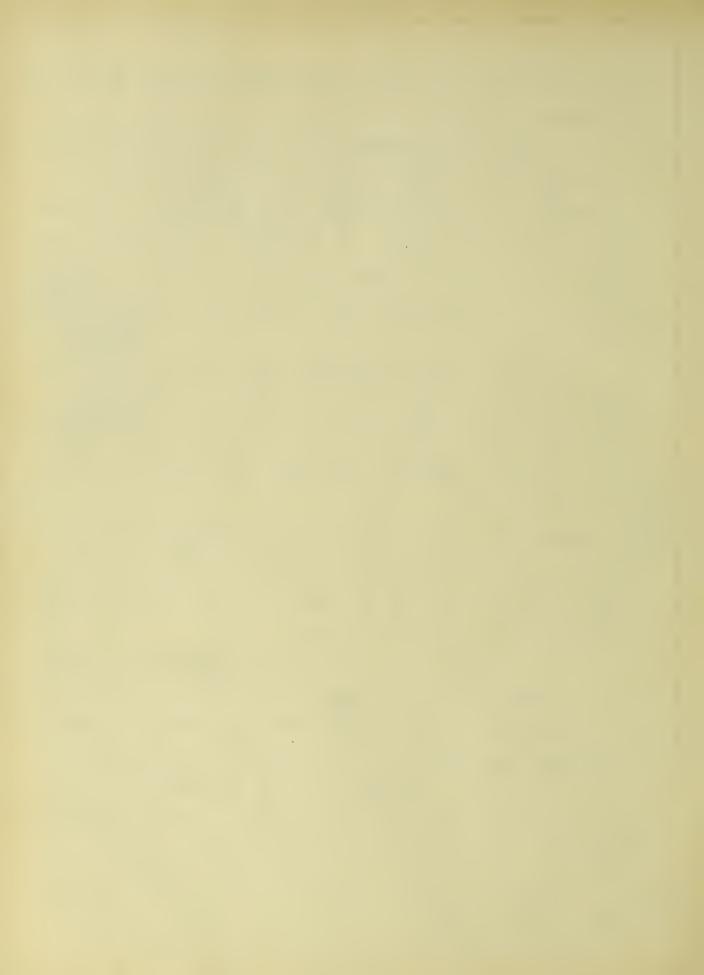
Teachydrolysis, but in the to more extensive costd of the suspension.

experiments and verifies the viriant in starch we have a supported and an emulation of the former totally out the latter. If no emulacid were resent, practically out the and immediate coandation while be expected in the solution containing more than an about of a strong electrolyte.

periments were some which cannot be reported. Difficulties were experienced in fixing the proper computations to be used in obtaining compared at a under the varying conditions of the experiences.

As the writer moves, no experient, are on recommendationed above, in first the very crass without or running the first out of a burelies of rased.

It is evident that for a study of the vincosity of starch the two components must be separated. If natural starch or the ordinary preparations of soluble starch are used, the presence of electrolytes makes the interpretation of the results practically impossible. It becomes very doubtful, therefore, whether a come in miscosity one at all be used as a measure of hydrolytic.



## BIBLIOGRAPHY.

- т. Постеон and Prence. A. Jann. Physiol. 35, 355 (1910).
- 2. Lee, Jour. Physiol. 11, 234 (1890).
- 2. Philoche, Jour. de Chimie de Janue, 6.
- 4. Armstron , Proc. Roy. Soc. 79, 360 (Ser. P).
- 5. Sanuely. Im Copenidance, Handbuch Jer Biocher Le des Menschen der Tiere (I 543) (1909).
- 7. V han, Intrazel disce Enzyme, Ergebnisse der Physiologie
  IX (1910)
- T. Euler Allmemeine Chemie der Enzyme, Ergebnisse er Physiologie IX (1930)
- 9. Oppenheimer, Die Fermante und ihre Wirkungen (1903)
- 9. Taylor, On Fermentation (page 83) University of California
  Publications (1907).
- 10. Hamburger, Pflüs. Arch. 60, 546 (1895)
- 17. Bierry et Giaja C.R. 143, 300 (1907)
- 12. Fier y et Torraine C. R. Soc. Biol. 59, 257 (1907).
- 13. Musculus and Mering, Ztc. offsiol. Chem. 2, 403 (1878)
- 4. Gedula, Wochenson ist " E suereiw ser 8, 545 (1891)
- 75. Cuisinier, Zts. . Ver. f. Zuch rriben industrie 23, 276 (1800) quoted according to Che . Zentr.
- 76. Woll, Per. 1, 2060 (1850).
- 17. Fischer, Ber. 27, 2895, (1895)
- 18. Röhmann, Ber. 27, 201 (1895)
- 11. Scrinder, Stand Arc. 1, 34 , (1837)
- 20. Grützner, Pflur Arch. 91, 195, (1902)



- 21. Ar henius, Zts. physik. Chem. 4, 266 (1899)
- 22. Posentheler, Pioc em. Zts. 26, 9 (1910)
- 13. Cole, Jam. of Pagelol. 20, 202 (1903)
- 74. Moore an Birland, Pincher. Jour. 5, 32 (1910)
- 25. Bierry and Terroine C. R. 141, 146 (1905)
- ff. Bierry and Glaia C. T. 12, CCC (1906)
- 27. Bierry and Ter oine C. J. Soc. I'd . J., 257
- TE. Bierry and Giaja C. R. Suc. Biol. 60, 749.
- o. Pierry C. R. Soc. Biol. 60, 1111
- 20. Biarry C. R. S.c. Biol. 62, 20
- 27. For an futhrie, Jun. Chem. Soc. 89, 76 (1906)
- 32. Trankel and Hamburn, Horm. Beitr. 8, 389 (1906)
- 23. Feris, The Nature of Engle action (1908)
- (1. Priti, Blochem. Zts. 4, J. (1907)
- 25. Wohlsemuth, Biocher Zto. 9, 1 (1908)
- 3. Woolmerath, Riocher Zts. 9, 10 (1908)
- 37. Aucoli and Fonfanti Zt., physiol. Chem. 43, 156 (1904)
- 38. Sherman, Kendall and Clark, J. A. C. S. 32, 1073 (1910)
- 35. Sherman Tr Kendall, J. A. C. S. 32, 1087 (1910)
- 40. C.a., F. C. Bull. 1, 194 (1911)
- 47. Starbenstein, rioc'em. Zts. 24, 191 (1910)
- 42 " " 24, 810 (1970)
- 45. B.c., Poù Ao. ..., 107 (1:00)
- n n se, 150 (1893)
- 15. II II II 54, 70 (7895)
- . Borcherit Piri- A . 100, 17 (1903)
- 77. Puliese. Pfi. Ar. CO, 11: (1898)



- . . Orich and Mortin, John . of Physiol. 40, 404 (190)
- 10. Min on and Walker, J. A. C. S. 24, 1 82 (1900)
- no. Prantite, Arch. . Kinderell le nee 41, 101
- :1. Simply, Z. . migrain. Ciem. 11, 200 (1898)
- 52. ylins, Its. Physiol. Cher. 17, 300 (1867)
- 50. " 7 ... 0, FEE (1857)
- F/. How ... Koll 3ts. 9, 1 1911)
- 5 -. Oct ald, Wo. Koll Plinefte (Hoft 8, J.)
- Dictour, Ar. Irat. I ... 8, 863 (1894)
- 57. Panic're, C'em. Ztr. 18, 157 (1894)
- E8. Joung, Jour. Physiol. 20, 401 (1893)
- 59. Kuster, Liet. Ann. 288, 360 (1895)
- 60. Göpplaröder, Pogg Ann. 119, 57 (1862)
- 61. Rouvier, C. R. 117, 401 (1898)
- 62. Maguenne and Roux, Ann. Chim. et Phys. 9, 179 (1908)
- 63. Fouard, C. R. 146, 285 (1908)
- C4 Gatin Gruzewska, C. R. 146, 540 (1908)
- 65. Manuere, C. R. 746, 277 (1908)
- 60. " C. R. 147, UMP (1908)
- 67. Ontweld, Wo. Grundrian der Kolloid chemie; 2nd chitton (1911).
- 67. Botto di in d'Errico, Pilur. Arc. 235, 359 (1906)
- 69. Ferniach and Volin, C. i. 142, 381 (1906)
- 70. Wortmann, Bot. Zt. No. 37 (quoted rom Oppenheimer)
- 71. Par. Ser Anderhaliant H. . Tipcium. Lielta colo e.





